Membrane targeting and intracellular trafficking of the human sodium-dependent multivitamin transporter in polarized epithelial cells

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BIOIN (vitamin H) is a water-soluble micronutrient required for normal cellular function, growth, and development in humans owing to its catalytic role in many biochemical reactions (6, 44). Biotin deficiency may result from inborn errors of biotin metabolism, long-term use of anticonvulsants, chronic alcoholism, or inflammatory bowel disease, in infants with seborrheic dermatitis and Leiner’s disease, and may occur during pregnancy (4, 5, 11, 21, 22, 27, 29, 33, 45). Humans have lost the capacity for de novo biosynthesis of biotin and thus must obtain the vitamin from dietary sources via intestinal absorption. In addition, systemic elimination of biotin is regulated via reabsorption of filtered biotin in the renal glomeruli. Thus renal and intestinal epithelial cells play pivotal roles in regulating biotin homeostasis. Biotin transport occurs via a carrier-mediated mechanism, mediated by the human sodium-dependent multivitamin transporter (hSMVT, the product of the SLC5A6 gene (36, 48)), the functional properties of which have been demonstrated in different epithelia (2, 3, 36, 41, 48, 49).

Materials. [1H]biotin (30 Ci/mmol, radiochemical purity >98%) was obtained from American Radiolabeled Chemical (St. Louis, MO). The enhanced green fluorescent protein (GFP-N3) and red fluorescent protein (DsRed) vectors and DsRed-endoplasmic reticulum (DsRed2-ER) were from Addgene (Cambridge, MA). Geneticin (G418) was from Invitrogen (Carlsbad, CA). Cytoskeletal disrupting agents were from Calbiochem (La Jolla, CA). The enhanced green fluorescent protein (GFP) fusion protein was functional and expressed at the apical membrane in renal and intestinal cell lines. Microtubule disrupting agents disrupted the mobility of trafficking vesicles and impaired cell surface delivery of hSMVT, which was also prevented in cells treated with dynamitin (p50), brefeldin, or monensin. Progressive truncation of the COOH-terminal tail impaired the functionality and targeting of the transporter. First, biotin transport decreased by approximately 20–30% on deletion of up to 15 COOH-terminal amino acids of hSMVT, a decrease mimicked solely by deletion of the terminal PDZ motif (TSL). Second, deletions into the COOH-terminal tail (between residues 584-612, containing a region of predicted high surface accessibility) resulted in a further drop in hSMVT transport (to ~40% of wild-type). Third, apical targeting was lost on deletion of a helical-prone region between amino acids 570-584. We conclude that the COOH tail of hSMVT contains several determinants important for polarized targeting and biotin transport.

MATERIALS AND METHODS

Materials. [1H]biotin (30 Ci/mmol, radiochemical purity >98%) was obtained from American Radiolabeled Chemical (St. Louis, MO). The enhanced green fluorescent protein (GFP-N3) and red fluorescent protein (DsRed) vectors and DsRed-endoplasmic reticulum (DsRed2-ER) and DsRed-Golgi plasmids were from BD Biosciences (Palo Alto, CA). Geneticin (G418) was from Invitrogen (Carlsbad, CA). Cytoskeletal disrupting agents were from Calbiochem (La Jolla, CA). Madin-Darby canine kidney (MDCK, NBL-2), human-derived duodenal adenocarcinoma cell line (HuTu-80), and human adenocarcinoma (Caco-2) cells were from American Type Culture Collection (ATCC). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Western blot analysis. Western blot analysis was performed on apical and basolateral membrane vesicles isolated from the human colon of organ donors using established procedures [kindly provided by Dr. Pradeep K. Dudeja, University of Illinois, Chicago, IL (10)]. Apical and basolateral membrane vesicles (30 μg) were heated with loading buffer (Invitrogen) for 5 min at 70°C and resolved onto premade 4–12% Bis-Tris Mini gel (Invitrogen). All samples were simultaneously run on the same gel. After electrophoresis, proteins were electroblotted onto polyvinylidene fluoride membrane (Bio-Rad, Hercules, CA). The membranes were washed with PBS containing 0.1% Tween 20 (Sigma) and then blocked with a PBS solution containing 5% dried milk (Bio-Rad) for 1 h at room temperature or overnight at 4°C. After blocking, membranes were incubated either with hSMVT polyclonal antibody raised in rabbit [Alpha Diagnostics, San Antonio, TX (32)] or with antigenic peptide (Alpha Diagnostics) pretested antibodies for 1 h at room temperature. Subsequently, membranes were washed twice and incubated with secondary antibodies [goat anti-rabbit conjugated to horseradish peroxidase (HRP; Santa Cruz Biotechnology, Santa Cruz, CA)]. Next, membranes were washed and incubated with enhanced chemiluminescent substrate (Amersham, Arlington Heights, IL) and exposed to radiography films. The open reading frame (ORF) of hSMVT (1,905 bp) was amplified by RT-PCR from human small intestinal total RNA using gene-specific primers (Table 1). The full-length hSMVT-GFP and truncated constructs were generated by PCR using the primer combinations shown in Table 1 and conditions previously described (40, 42). The PCR products and the GFP-N3 vectors were digested with the restriction enzymes EcoRI and Sal I, and the products were gel-separated and then ligated together to generate in-frame fusion proteins with the GFP fused to the COOH terminus of each construct. The nucleotide sequence of each construct was verified by sequencing (Laragen).

Table 1. Gene-specific primers used for generating hSMVT truncations

<table>
<thead>
<tr>
<th>Construct (Amino Acid)</th>
<th>Forward and Reverse Primers (5’-3’)</th>
<th>Positions, bp</th>
<th>Fragment, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>hSMVT-GFP</td>
<td>CCGGATTCATGAGTGTAGGGGTGAGC; ACGCGGACCCAGGAGGGCAGGCTCTAGGAG</td>
<td>1-1905</td>
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<tr>
<td>hSMVT[1-632]-GFP</td>
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<tr>
<td>hSMVT[1-620]-GFP</td>
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<td>1-1656</td>
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Shown is the combination of primer sequence used to generate full-length and truncated human sodium-dependent multivitamin transporter (hSMVT) constructs by PCR. Restriction sites for EcoRI (underlined) and Sal I (boldface) were added to the hSMVT primers to allow subsequent subcloning into the enhanced green fluorescent protein (GFP-N3) vector. hSMVT[ΔC]-truncation of COOH terminus of hSMVT.
Live cell imaging. Cells cultured on petri dishes were imaged using a Nikon C1 confocal scanner head attached to a Nikon inverted phase-contrast microscope. For imaging cells grown on filters, a Bio-Rad MRC 1024 confocal scanner attached to an Olympus Provis AX70 upright microscope equipped with a ×60 water immersion objective was used. Fluorophores were excited using the 488-nm line from an argon ion-laser, and emitted fluorescence was monitored with a 530-±20-nm band-pass (GFP) or a 620-nm long-pass filter (RFP). Total internal reflection fluorescence (TIRF) imaging of vesicular trafficking was performed using an Olympus TIRF illuminator attached to an IX70 inverted microscope. Images were captured using an EMCCD, Roper Cascade II, and the motion of individual vesicles tracked using a frame-to-frame tracking function in MetaMorph (Universal Imaging, Downingtown, PA). Videos are provided as Supplemental Movies S1–4 available in the data supplement online at the AJP-Cell Physiology web site.

Flow cytometry. Flow cytometry was performed using a FACS-Calibur bench-top cytometer (BD Biosciences). hSMVT wild-type and mutants transiently expressing MDCK cells were grown within T-25 tissue culture flasks. The monolayer was trypsinized, and cells were pelleted and resuspended in 1-ml aliquots of Ca²⁺- and Mg²⁺-containing HBSS as described previously (39). In all flow cytometry experiments, samples of untransfected and GFP alone transfected MDCK cells were run in parallel with experimental samples to calibrate optical parameters for identifying the intact, transfected cell population.

RESULTS

hSMVT-GFP targeting and functionality in polarized epithelia. A schematic representation of the full-length hSMVT-GFP fusion construct is shown in Fig. 1A. This representation depicts the overall organization of the polypeptide (635 amino acids), which includes a short cytoplasmic NH₂-terminal domain (residues 1-24), a transmembrane domain with 12 predicted membrane spanning regions (residues 25-552), and a cytoplasmic COOH-terminal domain (83 amino acids, residues 553-635) to which GFP was attached (Fig. 1A). To examine the targeting and functionality of the full-length hSMVT protein, a stable hSMVT-GFP-expressing MDCK cell line was generated (see MATERIALS AND METHODS). Semiquantitative RT-PCR demonstrated hSMVT-mRNA expression was ~9-fold higher in the stable cell line than mock-transfected MDCK cells (data not shown). Confocal analysis of confluent monolayers grown

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Fig. 1. Apical targeting of the human sodium-dependent multivitamin transporter (hSMVT)-green fluorescent protein (GFP) fusion protein in renal and intestinal epithelial cells. A: schematic representation of the full-length hSMVT protein (1-635 residues) with GFP fused to the COOH terminus (hSMVT-GFP). B: distribution of hSMVT-GFP and GFP alone in lateral (xy, left) and axial (z; right) section in renal [top; Madin-Darby canine kidney (MDCK)] and intestinal [bottom; human adenocarcinoma (Caco-2)] cell lines. MDCK cells were cotransfected with red fluorescent protein (DsRed) vector to allow resolution of cellular volume. Scale bar is 5 μm. C: [³H]biotin uptake assays in a stable hSMVT-GFP-expressing MDCK cell line grown 5–7 days after confluence on permeable filter supports after introduction of [³H]biotin to the apical (solid) or basolateral (open) chamber. D: expression of the hSMVT protein in native human colon apical membrane vesicles (AMV) and basolateral membrane vesicles (BLM) by Western blotting. All samples were run simultaneously on the same gels (but lanes were grouped for clear presentation), and a representative blot is shown. I: membranes were incubated with primary polyclonal anti-hSMVT antibodies (Ab) raised in rabbits and horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-rabbit). II: membranes were incubated with only secondary antibodies. III: anti-hSMVT antibodies pretreated first with antigenic peptide and then incubated with secondary antibodies. Bottom are the same membranes stripped and incubated with human β-actin antibodies. Molecular mass estimations (in kilodaltons) are shown.
on filter supports revealed apical targeting of hSMVT-GFP (Fig. 1B). Similar results were obtained with filter-grown confluent Caco-2 cells (Fig. 1B). In contrast, expression of GFP alone (as a control) resulted in cytoplasmic fluorescence in both MDCK and Caco-2 cells (Fig. 1B). To determine the functional consequences of the asymmetrical distribution of hSMVT-GFP, [3H]biotin was introduced either to the apical or basolateral compartment. Apical [3H]biotin uptake increased ~40-fold in hSMVT-GFP-expressing stable MDCK cells compared with mock-transfected cells, whereas there was no significant change in basolateral uptake of [3H]biotin in stable compared with mock-transfected MDCK cells (Fig. 1C). Finally, expression of hSMVT was assessed in native human colon by Western blotting, using enriched colonic apical and basolateral membrane preparations isolated from organ donors by established procedures (10) and well-characterized polyclonal anti-hSMVT antibodies (32). The hSMVT protein was predominantly expressed in the apical membrane preparation (Fig. 1D). Taken together, the asymmetry in [3H]biotin uptake was consistent with confocal imaging and immunoblotting data, underscoring the concept that hSMVT mediates biotin transport across the apical domain of polarized epithelia.

Cytoplasmic COOH-terminal truncations impact [3H]biotin uptake. To assess the role of the cytoplasmic COOH tail of hSMVT, we sequenced a series of 10 truncation constructs in the tail region (Table 1). Each construct was analyzed in terms of (1) functionality ([3H]biotin uptake), and (2) population expression level of the truncated construct (>10,000 cells, flow cytometry analyses). A crude way of interpreting the functionality of each of these constructs was obtained by calculating the level of [3H]biotin transport normalized to the expression level of each construct (judged by means of population fluorescence), i.e., the rate of transport relative to the amount of hSMVT expressed (Fig. 2). Each construct was then further analyzed at the single cell level to resolve the subcellular localization of each mutant. These analyses are discussed, in turn, below.

From the collated results shown in Fig. 2, it is clear that truncation of the entire cytoplasmic COOH tail (amino acids 553–635, hSMVT[ΔC]-GFP) (truncation of COOH terminus of hSMVT) abrogated hSMVT functionality, as no enhancement of [3H]biotin uptake was observed relative to controls. However, functionality was recovered in constructs with shorter deletions. Truncation mutants fell into 2 broad groupings, exhibiting ~75% or approximately 25–50% of wild-type activity. The grouping of higher activity (group 1) was associated with deletions up to 15 residues (up to residue 620, i.e., 621-635 deleted), encompassing constructs hSMVT[632]-GFP, hSMVT[624]-GFP, and hSMVT[620]-GFP. The next broad grouping (group 2) encompassed truncations of up to 60 residues (up to residue 575), encompassing hSMVT[612]-GFP, hSMVT[600]-GFP, hSMVT[584]-GFP, and hSMVT[575]-GFP. Further deletions resulted in a more progressive loss of functionality, and constructs hSMVT[ΔC]-GFP, hSMVT[567]-GFP, and hSMVT[570]-GFP were all transport-null (group 3). Therefore, these experiments implicated 3 broad regions in the COOH tail of hSMVT that progressively impacted cellular [3H]biotin accumulation (Fig. 2).

We proceeded to investigate the polarized targeting of these 10 constructs in both MDCK and Caco-2 cells. Each truncated construct was transiently transfected into MDCK and Caco-2 cells, and the resulting cellular distribution was resolved by confocal imaging (Fig. 3). The 3 constructs that displayed slight impaired functionality (group 1; Fig. 2), namely hSMVT[632]-GFP, hSMVT[624]-GFP, and hSMVT[620]-GFP, all targeted to the apical cell surface, just like the full-length transporter (Fig. 3A). The next group of deletions associated with a further impairment in [3H]biotin transport (group 2; Fig. 2), namely hSMVT[612]-GFP, hSMVT[600]-GFP, hSMVT[584]-GFP, and hSMVT[575]-GFP, targeted to the apical cell surface but displayed a progressively increased punctate intracellular fluorescence suggestive of an increased redistribution to trafficking vesicles (Fig. 3B). Finally, no cell surface expression was observed with hSMVT[570]-GFP, hSMVT[567]-GFP, and hSMVT[ΔC]-GFP, rather fluorescence expression was confined within intracellular membranes (Fig. 3C), consistent with their behavior as transport-null constructs (group 3; Fig. 2). In cells cotransfected with hSMVT[570]-GFP and an ER-targeted red fluorescent protein construct (DsRed2-ER), significant fluorescence overlap was observed. This contrasted with cells cotransfected with DsRed2-ER and, for example, hSMVT[620]-GFP (Fig. 3D). In summary, group 1 constructs displayed impaired functionality but normal apical targeting, group 2 constructs displayed progressively impaired apical targeting and decreased functionality, whereas group 3 constructs were retained within the ER.

Altered targeting of the COOH tail of hSMVT. As presented above, truncation of the COOH-terminal tail of hSMVT alters cell surface expression. Surprisingly, the serial truncation analysis resulted in the identification of one construct (hSMVT[616]-GFP) that displayed a mispolarized cell surface expression profile with marked basolateral expression in both MDCK and Caco-2 cells (Fig. 4). Additionally, [3H]biotin uptake by confluent monolayers expressing this truncated construct, i.e., uptake across the apical membrane domain, was decreased.

![Fig. 2. Functionality of hSMVT COOH-terminal tail truncations. Functionality of 10 cytoplasmic tail truncations relative to that of full-length hSMVT-GFP was calculated by measuring the amount of [3H]biotin uptake in a defined period (3 min) corrected for the construct expression level (judged by the mean population fluorescence by flow cytometry). The length of the construct is indicated on the x-axis (amino acids). Data are corralled into 3 groups relative to wild-type (■, 635 amino acids): ~75% wild-type functionality (○, group 1), approximately 25–50% wild-type functionality (●, group 2), and transport-null constructs (○, group 3).](http://ajpcell.physiology.org/)

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compared with wild-type (226 ± 7 vs. 86 ± 8 fmol/mg protein/3 min for wild-type and hSMVT[616]-GFP, respectively), which is most likely due to the decrease in apical expression of the truncated protein in transfected cell monolayers.

**Microtubule-dependent apical expression of hSMVT.** The role of the microtubule network in the apical targeting of hSMVT was investigated. To examine the effect of microtubule disruption on steady-state expression of hSMVT, confluent MDCK cells were treated with nocodazole (20 μM, 30 min), after which cells were transfected with hSMVT-GFP cDNA before imaging 18–24 h later (as described previously in Ref. 23). Nocodazole treatment impaired the apical localization of hSMVT, yielding a nonpolarized distribution of hSMVT-GFP and a significant increase in intracellular fluorescence resolved in axial confocal sections (Fig. 5A). In contrast, treatment with cytochalasin did not impair the apical targeting of hSMVT-GFP (10 μM, 30 min). These results suggested that apical expression/retention of hSMVT in MDCK cells was dependent on an intact microtubule network.

To resolve more acute effects of cytoskeletal disruption on the real-time trafficking of full-length hSMVT-GFP, we used TIRF microscopy to image the behavior of individual vesicles near the cell surface. For the TIRF experiments, we used a human duodenally derived cell line (HuTu-80). Usage of this particular cell line facilitated trafficking studies as 1) vesicles are numerous, and 2) the cell line does not show polarized transporter expression under our culture conditions, enabling vesicle tracking at cell/cover glass interface.

A stable hSMVT-GFP-expressing HuTu-80 cell line displayed fluorescence both at the cell surface as well as within numerous cytoplasmic structures (Fig. 5B, i). Functional measurements of [3H]biotin accumulation indicated that [3H]biotin uptake was ~5-fold greater in the stable cell line, confirming the functionality of the full-length fusion protein (Fig. 5B, ii). Densitometric quantification of RT-PCR products demonstrated that hSMVT expression was ~4.5-fold greater in the stable cell line compared with mock-transfected cells (Fig. 5B, ii, inset).

TIRF imaging of the near-membrane dynamics of hSMVT-GFP resolved considerable vesicular motility within the evanescent field (Fig. 5C, i; Supplemental Movie S1). Examples of motion comprised transitions into and out of the TIRF field.
hSMVT is a Na⁺-dependent, electrogenic biotin transporter that is responsible for apical biotin uptake in polarized epithelia (26, 36). Expression of hSMVT is subject to adaptive regulation under scenarios of biotin deficiency (3, 37). Here, we used live cell imaging and [³H]biotin uptake approaches to investigate the cellular mechanisms governing the trafficking and targeting of this transporter in a variety of epithelial cell lines. Serial truncations into the COOH terminus of hSMVT delineated several domains within the cytoplasmic tail important for biotin transport and ultimately export of the transporter from the ER to the apical cell surface. First, deletions of up to 15 amino acids (group 1) resulted in ≤25% decrease in the rate of biotin transport (Fig. 2), without impairment of plasma membrane targeting in polarized renal and intestinal epithelial cells. This region of the cytoplasmic tail is predicted to terminate with an extended confirmation ending with a consensus PDZ motif as seen in many transporters (1, 7, 12, 28). This is of interest given that deletion of only the 3 terminal amino acids of hSMVT (TSL) comprising the PDZ motif (28) was sufficient to decrease the rate of [³H]biotin accumulation. The next set of truncations (group 2) contains a region predicted in silico to have high surface accessibility (35); deletions into this next set of truncations (group 2) contained the localization of 12–15% decrease in biotin transport, compared with polarized apical expression in control cells (Fig. 6B).

Apical hSMVT targeting occurs via a BFA- and monensin-sensitive pathway. BFA is a fungal metabolite known to cause the Golgi to fuse with ER and block intracellular vesicular transport to the cell surface (8, 20, 25, 34, 43). Monensin also blocks the delivery of newly synthesized membrane protein to the cell membrane (13, 30, 34, 43). Both drugs disrupted apical expression of hSMVT in the stable MDCK cell line. BFA (5 μg/ml, 14 h at 37°C) caused hSMVT-GFP to accumulate in the ER as demonstrated by colocalization with DsRed2-ER in the majority of cells (Fig. 6A). In a minority of cells (approximately 10–15%), a nonpolarized distribution across the cell surface was evident (data not shown). Monensin (5 μM, 14 h at 37°C) blocked the delivery of hSMVT to the apical membrane, and hSMVT remained within intracellular structures (Fig. 6C). Again, in a small fraction of cells (approximately 10–15%), a nonpolarized distribution at the cell surface was observed. Taken together, these results suggest that the apical targeting of hSMVT-GFP is through a classic BFA- and monensin-sensitive pathway.

DISCUSSION

hSMVT is a Na⁺-dependent, electrogenic biotin transporter that is responsible for apical biotin uptake in polarized epithelia (26, 36). Expression of hSMVT is subject to adaptive regulation under scenarios of biotin deficiency (3, 37). Here, we used live cell imaging and [³H]biotin uptake approaches to investigate the cellular mechanisms governing the trafficking and targeting of this transporter in a variety of epithelial cell lines. Serial truncations into the COOH terminus of hSMVT delineated several domains within the cytoplasmic tail important for biotin transport and ultimately export of the transporter from the ER to the apical cell surface. First, deletions of up to 15 amino acids (group 1) resulted in ≤25% decrease in the rate of biotin transport (Fig. 2), without impairment of plasma membrane targeting in polarized renal and intestinal epithelial cells. This region of the cytoplasmic tail is predicted to terminate with an extended confirmation ending with a consensus PDZ motif as seen in many transporters (1, 7, 12, 28). This is of interest given that deletion of only the 3 terminal amino acids of hSMVT (TSL) comprising the PDZ motif (28) was sufficient to decrease the rate of [³H]biotin accumulation. The next set of truncations (group 2) contains a region predicted in silico to have high surface accessibility (35); deletions into this region resulted in a further ~25% decrease in biotin transport, possibly attributable to the decreased localization of transporters at the cell surface. Interestingly, the region between these domains (groups 1 and 2) contained the localization of 12–15% decrease in biotin transport, compared with polarized apical expression in control cells (Fig. 6B).

Apical hSMVT targeting occurs via a BFA- and monensin-sensitive pathway. BFA is a fungal metabolite known to cause the Golgi to fuse with ER and block intracellular vesicular transport to the cell surface (8, 20, 25, 34, 43). Monensin also blocks the delivery of newly synthesized membrane protein to the cell membrane (13, 30, 34, 43). Both drugs disrupted apical expression of hSMVT in the stable MDCK cell line. BFA (5 μg/ml, 14 h at 37°C) caused hSMVT-GFP to accumulate in the ER as demonstrated by colocalization with DsRed2-ER in the majority of cells (Fig. 6A). In a minority of cells (approximately 10–15%), a nonpolarized distribution across the cell surface was evident (data not shown). Monensin (5 μM, 14 h at 37°C) blocked the delivery of hSMVT to the apical membrane, and hSMVT remained within intracellular structures (Fig. 6C). Again, in a small fraction of cells (approximately 10–15%), a nonpolarized distribution at the cell surface was observed. Taken together, these results suggest that the apical targeting of hSMVT-GFP is through a classic BFA- and monensin-sensitive pathway.
and a corresponding marked retention of fluorescence with intracellular vesicles (hSMVT[575]-GFP) and ultimately the ER (Fig. 3). The elimination of transport on removal of the 5 residues between hSMVT[575]-GFP and hSMVT[570]-GFP corresponds to deletions into a predicted helical region, which extends upstream to encompass a putative endocytosis motif (YXXL) as well as a polyproline core (PXXP) and 2 sequential dileucine (LL) motifs that have been implicated as signals important for targeting in several other transporters (14, 16, 18). Future studies will examine the role of these regions in the context of the full-length transporter.

Equilibrium and real-time trafficking analyses implicated a crucial role for microtubule-based processes in cell surface delivery of hSMVT-GFP. Acute and chronic treatment with nocodazole impaired vesicular motility and cell surface delivery, respectively, whereas microfilament disruption had little apparent effect. The dynamics of hSMVT-containing structures observed in epithelial cells is of obvious interest in connection with potential regulation of transporter levels at the cell surface that may take place under certain conditions. One of several mechanisms that can be envisaged to impact overall nutrient uptake capacity by a given cell would be differential rates of transporter insertion/retrieval from the apical membrane domain as has been shown for a variety of substrates (15, 17, 38). The ability to resolve individual hSMVT vesicle trafficking events (Fig. 5) will allow future studies to evaluate whether regulation of transporter insertion and retrieval occurs in cells under specific conditions. Apical membrane localization of hSMVT is mediated at least in part by BFA-sensitive pathway consistent with other apical membrane targeting proteins (8, 25, 43).
In conclusion, we have demonstrated that domains within the COOH-terminal tail of hSMVT are essential for functionality of the hSMVT transporter and for expression at the apical plasma membrane domain in both renal and intestinal epithelia.

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