A Human Homolog Can Functionally Replace the Yeast Vesicle-associated SNARE Vti1p in Two Vesicle Transport Pathways*

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Membrane traffic in eukaryotic cells requires the interaction of a vesicle-associated soluble NSF attachment protein receptor (v-SNARE) on transport vesicles with a SNARE on the target membrane (t-SNARE). Recently, we identified the yeast protein Vti1p as a v-SNARE that is involved in two transport reactions. Vti1p interacts with the prevacuolar t-SNARE Pep12p in Golgi to prevacuolar transport and with the cis-Golgi t-SNARE Sed5p in traffic to the cis-Golgi. Here we describe a human Vti1 homolog, hVti1. Whereas vti1Δ cells are inviable, expression of hVti1 allows vti1Δ cells to grow at nearly the wild-type growth rate. When expressed in yeast hVti1 can replace Vti1p as a v-SNARE that is involved in two transport reactions. Vti1p interacts with the prevacuolar t-SNARE Pep12p in Golgi to prevacuolar transport and with the cis-Golgi t-SNARE Sed5p in traffic to the cis-Golgi. Sequence comparisons with a Schizosaccharomyces pombe and two different mouse Vti1 homologs led to the identification of a very conserved predicted α-helix. Amino acid exchanges in vti1 mutant alleles defective either in one or both trafficking steps cluster in this domain, suggesting that this structure is probably the binding site for effector proteins.

Transport between many different organelles in eukaryotic cells occurs via transport vesicles, which must have the ability to recognize their target membranes. SNARE proteins provide this information (1, 2). In the SNARE model, a specific set of v-SNAREs localized on transport vesicles interacts with specific t-SNAREs on the target membrane. Both v- and t-SNAREs contain a single C-terminal transmembrane domain and predicted coiled coil domains. It has been demonstrated that t-SNAREs interact via their coiled coil domains with v-SNAREs. A growing number of SNARE proteins have been identified from both yeast and mammalian cells. SNARE proteins involved in identical membrane trafficking steps in yeast and mammals share significant amino acid identity. In yeast the v-SNAREs Sec22p (Sly2p), Bet1p (Sly12p), Bos1p, and Ykt6p are involved in transport from the ER to the cis-Golgi compartment (3–5). Their t-SNARE partner in the cis-Golgi compartment is Sed5p (6). It has been demonstrated that in addition to interactions with the anterograde v-SNAREs, Sed5p also binds to the medial Golgi v-SNARE Ssl1p, which is involved in retrograde traffic from the medial to the cis Golgi compartment (7). Recently, Sec22p has been found in a complex with the ER t-SNARE Ufe1p and has been implicated in retrograde traffic to the ER (8). Mammalian homologs have been identified for Sec22p, rsec22 and ERS-24, and for Bet1p, rbet1 (9, 10). ERS-24 interacts with syntaxin 5, the mammalian homolog of Sed5p (11).

Proteins traversing the secretory pathway are sorted in the trans-Golgi network according to their destination (12). In mammalian cells, vesicles of the constitutive and regulated secretory pathway destined for the plasma membrane bud from the trans-Golgi network. Soluble lysosomal proteins are marked by a mannose 6-phosphate residue and bind to the mannose 6-phosphate receptor (13). The complex leaves in transport vesicles targeted to the late endosomal compartment, from which the mannose 6-phosphate receptor is recycled. In yeast about 50 VPS and PEP genes have been identified, which function in traffic from the late Golgi compartment to the vacuole, the mammalian equivalents of the trans-Golgi network and the lysosome respectively (14–17). The soluble vacuolar hydrolase carboxypeptidase Y (CPY) binds to the CPAI receptor Vps10p in the late Golgi compartment via a peptide sorting signal (18). The complex is transported to the prevacuolar compartment where it dissociates. CPY is transported on to the vacuole and Vps10p is recycled (19–21). Pep12p has been identified as a t-SNARE residing in the prevacuolar compartment (22). The mammalian protein syntaxin 6 displays 25% amino acid identity with Pep12p, is localized in the Golgi region, and has been proposed to be the functional homolog of Pep12p (23). A Pep12p homolog with 32% amino acid identity has been identified in Arabidopsis, which can restore at least some vacuolar protease function to pep12 mutants in yeast (24).

Recently, we described a yeast v-SNARE, Vti1p, that is essential for yeast cell viability (25). The temperature-sensitive vti1 mutants vti1-1 and vti1-2 are defective in late Golgi to prevacuolar transport of CPY. Genetic interactions between VTI1 and PEP12 as well as physical association of Vti1p and Pep12p indicate that Vti1p and Pep12p form a v-SNARE-t-SNARE complex. A second class of temperature-sensitive mutants, such as vti1-1, display a severe growth defect and a block in traffic to the cis-Golgi compartment, in addition to a block in late Golgi to prevacuolar transport of CPY. Overexpression of Sed5p suppressed the cis-Golgi traffic block but had no effect on the defect in sorting proteins to the vacuole. Recombinant Vti1p and Sed5p interacted in vitro. We have proposed that Vti1p forms a SNARE complex with Sed5p in retrograde traffic either from the prevacuolar compartment or from the late Golgi compartment.

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‡ The abbreviations used are: SNARE, soluble NSF attachment protein receptor; v-SNARE, vesicle-associated SNARE; t-SNARE, target membrane-associated SNARE; CPY, carboxypeptidase Y; ER, endoplasmic reticulum; EST, expressed sequence tag; PCR, polymerase chain reaction; kb, kilobase pair(s).
Here we describe the identification of a human Vti1 homolog, hVti1, that can function in yeast. Conserved amino acid residues between the yeast and human proteins together with mutations in yeast VTI1 have led us to propose a structural model, in which a predicted α-helical coiled coil domain in Vti1p interacts with both Pep12p and Sed5p.

EXPERIMENTAL PROCEDURES

**Materials**—Reagents were used from the following sources: enzymes for DNA manipulation from New England Biolabs (Beverly, MA) and Boehringer Mannheim, [35S]Express label from NEN Life Sciences Products, fixed Staphylococcus aureus cells (IgGor) from The Enzyme Center (Malden, MA), and Oxalylcysteine from Enzogenetics (Corvallis, OR). The human hypothalamus cDNA library in λZAPII was obtained from the ATCC (Rockville, MD). All other reagents were purchased from Sigma. Yeast strains (Table I) were grown in rich medium (1% yeast extract, 1% peptone, 2% dextrose, YEPD) or standard minimal medium (SD) with appropriate supplements. To induce expression of the GAL1 promoter, dextrose was replaced by 2% raffinose and 2% galactose. Plasmid manipulations were performed in the Escherichia coli strains MC1061 or XL1Blue using standard media (Table II).

**Plasmids and Strains**—hVti1 was PCR-amplified from a human hypothalamus cDNA library in λZAPII as described above. The fragment was gel purified, digested with SacI and XhoI, and cloned into pBluescript KS⁺ to obtain pFVM6. To isolate pFVM50 and pFVM61, hVti1 was PCR-amplified from a human hypothalamus cDNA into λZAPII (27) using a T7 oligonucleotide and the same 3'-helical coiled coil domain in Vti1p interacts with both Pep12p and Sed5p.

**RESULTS**

**Identification of a Human Vti1p Homolog**—A multicopy suppressor screen was used to identify human proteins that allow for survival of yeast cells in the absence of Vti1p. The pADANS library contains human globlastoma cDNAs fused to the ADH1 promoter and the first 14 ADH1 codons, which results in expression of human fusion proteins in yeast (26). In the resulting transformants, expression of VTI1 was turned off by plating the cells on media containing glucose. Colonies that grew were tested for the absence of Vti1p by immunoblot analysis. Plasmids were recovered from these suppressor strains, and retransformed into vti1Δ GAL-VTI1 cells. Colonies that lost the GAL1-VTI1 plasmids were selected to confirm that suppression was dependent on the expression of a human protein. Sequencing of the suppressor (pFVM50) revealed that the clone is predicted to encode a 232-amino acid protein with a C-terminal transmembrane domain. This protein displays 29% overall amino acid identity with the yeast Vti1p (25), and was therefore called hVti1 (Fig. 1A). The 56-amino acid domain adjacent to the transmembrane domain is more homologous (41% amino acid identity). The regions between amino acid 38 and 67 and amino acid 160 and 193 are predicted to form amphipathic α-helical coiled coils using the paircoil program (probability score 0.71 and 0.69) (34). The region in yeast Vti1p homologous to the second predicted coiled coil region is also predicted to form a coiled coil domain, indicating that the proteins may adopt similar structures. The first structural studies with SNARE proteins indicate that the isolated t-SNAREs SNAP-25 and Sec9p and the v-SNARE Snc1p are largely unstructured (35, 36). A large increase in α-helical contents was observed after formation of SNARE complexes. Complex formation may induce similar structural changes in Vti1p.

By searching a data base of human expressed sequence tags (EST), human sequences that show high homology to the C-terminal half of the yeast Vti1p were identified (accession nos. N93765 and R88750). These sequences were identical to the C terminus of Vti1p identified in the suppressor screen. An oligonucleotide primer complimentary to sequences 3' of the Vti1p and a primer containing vector sequences around the polylinker were used to PCR amplify the hVti1 from two different human cDNA libraries. One library consisted of human globlastoma cDNA in the yeast expression plasmid pADANS (26). The other was human hypothalamus cDNA inserted into λZAPII phage (27). DNA sequences were amplified from both libraries, and these were predicted to encode a 171 amino acid

<table>
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<tr>
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<td>MATα leu2-3,112 ura3-52 ade2-101 trp1Δ-Δ901 suc2Δ-Δ mel- vti1Δ::His3</td>
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<td>FvMY7</td>
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**Table I**

**Yeast strains used in this study**

For CPY immunoprecipitations yeast cells were grown at 22 or 30 °C, radiolabeled with [35S]methionine for 10 min at the indicated temperature, and chased for 30 min after addition of 500 μg/ml methionine and cysteine. Invertase was derepressed by an incubation in minimal medium containing 0.1% glucose, 50 mM KPO₄, pH 5.7, and 1 mg/ml bovine serum albumin for 30 min at 22 °C plus 15 min at 37 °C. Cells were radiolabeled for 7 min at 37 °C and chased for 0 min or 30 min. Cells were spheroplasted, and extracts were prepared from internal and external fractions, and CPY- or invertase-immunoprecipitated with polyclonal antibodies and fixed for autoradiography.

**Human v-SNARE hVti1 Is Functional in Yeast**

This study uses a T7 oligonucleotide and the same 3'-helical coiled coil domain in Vti1p interacts with both Pep12p and Sed5p.

**Screen for Human vti1 Suppressors**—To identify human proteins that allow for growth in the absence of Vti1p, the strain FvMY8/ pFVM16 (vti1Δ:pCEN-GAL1-VTI1) was used. FvMY6/pFVM16 cells were transformed with yeast expression plasmids encoding human proteins. This library consisted of human globlastoma cDNAs fused to the ADH1 promoter and the first 14 ADH1 codons in the multicopy yeast vector pADANS (26). Transformants were plated on SD-Leu plates, conditions that turn off the expression of VTI1 and prevent growth of FvMY6/pFVM16 cells because Vti1p is essential for growth. Colonies that lost the expression of Vti1p by immunoblot analysis of the resulting transformants, expression of VTI1 was turned off by plating the cells on media containing glucose. Colonies that grew were tested for the absence of Vti1p by immunoblot analysis. Plasmids were recovered from these suppressor strains, and retransformed into vti1Δ GAL-VTI1 cells. Colonies that lost the GAL1-VTI1 plasmids were selected to confirm that suppression was dependent on the expression of a human protein. Sequencing of the suppressor (pFVM50) revealed that the clone is predicted to encode a 232-amino acid protein with a C-terminal transmembrane domain. This protein displays 29% overall amino acid identity with the yeast Vti1p (25), and was therefore called hVti1 (Fig. 1A). The 56-amino acid domain adjacent to the transmembrane domain is more homologous (41% amino acid identity). The regions between amino acid 38 and 67 and amino acid 160 and 193 are predicted to form amphipathic α-helical coiled coils using the paircoil program (probability score 0.71 and 0.69) (34). The region in yeast Vti1p homologous to the second predicted coiled coil region is also predicted to form a coiled coil domain, indicating that the proteins may adopt similar structures. The first structural studies with SNARE proteins indicate that the isolated t-SNAREs SNAP-25 and Sec9p and the v-SNARE Snc1p are largely unstructured (35, 36). A large increase in α-helical contents was observed after formation of SNARE complexes. Complex formation may induce similar structural changes in Vti1p.

By searching a data base of human expressed sequence tags (EST), human sequences that show high homology to the C-terminal half of the yeast Vti1p were identified (accession nos. N93765 and R88750). These sequences were identical to the C terminus of Vti1p identified in the suppressor screen. An oligonucleotide primer complimentary to sequences 3' of the Vti1p and a primer containing vector sequences around the polylinker were used to PCR amplify the hVti1 from two different human cDNA libraries. One library consisted of human globlastoma cDNA in the yeast expression plasmid pADANS (26). The other was human hypothalamus cDNA inserted into λZAPII phage (27). DNA sequences were amplified from both libraries, and these were predicted to encode a 171 amino acid
protein starting at methionine 62 of hVti1 (Fig. 1A, hVti1 starting at the arrowhead). A clone derived from the λ library (pFvM61) had an in-frame stop codon upstream of the putative initiating methionine 62. A different λ clone (pFvM60) and the clone derived from the pADANS library (pFvM 46) contained a coding region for the same 171-amino acid long hVti1 but were assembled from a second set of mouse ESTs. Unfortunately, the available sequences do not include the stop codon and end directly before the expected transmembrane domain. mVti1b shares only 31% amino acid identity with hVti1 and about the same degree of amino acid identity with S. cerevisiae Vti1p (35%).

Northern blot analysis of human RNA from different tissues revealed that hVti1 was expressed as a single band of about 1.2 kb in all tissues (data not shown). This suggests that hVti1 has a role in basic cell function.

Further data base searches revealed the presence of a Vti1-related hypothetical protein of unknown function in Schizosaccharomyces pombe, SpVti1 (Fig. 1A), which shares 38% amino acid identity with S. cerevisiae Vti1p (Fig. 1B). Recently, several Vti1p-related mouse ESTs were entered into the data base. The ESTs were assembled into a mouse Vti1 protein (mVti1), which is almost identical to hVti1 (93% amino acid identity). Surprisingly, a second Vti1-related protein, mVti1b, could be assembled from a second set of mouse ESTs. Unfortunately, the available sequences do not include the stop codon and end directly before the expected transmembrane domain. mVti1b shares only 31% amino acid identity with mVti1 and about the same degree of amino acid identity with yeast Vti1p (35%).

Three human ESTs with high identities to mVti1b were found in the data base (AA326353, R929052, and T70362), indicating the presence of a hVti1b, but the sequence data are not extensive enough to assemble the full protein.

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**Table II**

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<td>1.8 kb containing VTI1 in pRS314 (CEN6-TRP1)</td>
<td>Fischer von Mollard et al. (25)</td>
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<tr>
<td>pFvM46</td>
<td>PCR-amplified hVti1 from pADANS in pBluescript KS+</td>
<td>Fischer von Mollard et al. (25)</td>
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<td>pADANS vti1A suppressor encoding hVti1</td>
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<td>pFvM61</td>
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**Fig. 1.** Sequence comparison between different Vti1 proteins. A, different Vti1 proteins were aligned using the ClustalW program (50). The transmembrane domains are boxed, predicted coiled coil regions marked with a solid line (higher probability) or dashed line (lower probability) above the sequence. Arrowhead, start of the short hVti1; predicted fusion peptides between solid circles; conserved predicted α-helix between solid squares; amino acid exchanges in yeast mutant Vti1 proteins: *, vti1-1; +, vti1-2; Δ, vti1-11; ◊, vti1-12. Accession nos.: HVti1, AF033824; SpVti1, D89116; MVTI1, AA240967, AA016651, AA008789, AA105524, AA013839, AA086695, G1058685, W53760, and W94286; MVTI1b, AA097517, AA016654, AA016379, and W13616. B, amino acid identities and amino acid similarities taking conserved exchanges into account. The GAP program was used for pairwise comparison.
of vti1 Mutants—In Vti1p the domain between amino acid 37 and 60 is predicted to form an amphipathic α-helix which contains charged amino acids on one side and the other face is strongly hydrophobic due to the presence of bulky hydrophobic amino acids (Fig. 2A). The homologous domains in the other Vti1p-like proteins (Fig. 1, between filled circles) also display similar properties. In hVti1 and mVti1 the hydrophobic face is less pronounced but the domain between amino acid residues 77 and 98 can also form an amphipathic α-helix. These domains resemble amphipathic α-helical fusion peptides found in viral fusion proteins and are also present close to the N terminus in other v-SNAREs (37–39).

An alignment of all Vti1 proteins revealed a domain of 75 amino acids next to the transmembrane domain, which could be aligned without gaps and shows blocks of high amino acid identity. Within this domain all Vti1 proteins contain amino acid stretches that are predicted to form amphipathic α-helical coiled coils with high probability (Fig. 1, solid lines above the sequences) and lower probability (dashed lines). Using the paircoil program (34) the probability scores were: hVti1 0.69, mVti1 0.74, mVti1b 0.30, SpVti1 0.85, and Vti1 0.29. Coiled coil formation may require interaction with other proteins as observed for SNAP-25, Sec9p, and the v-SNARE Snc1p (35, 36). To align sequences from different species, we used the Maxhom multiple sequence alignment program (40), and the secondary structure of the alignment predicted with the EMBL PHDsec program (41). The domain between amino acids 132 and 190 in the Vti1 alignment is predicted to be α-helical with a very high probability. To visualize conserved features, the Vti1 alignment between amino acids 132 and 190 was drawn as a helical wheel projection (Fig. 2A). Amino acid residues that were identical in all Vti1 proteins were depicted in uppercase letters. Uppercase letters represent a conserved R or K. X marks residues that are not conserved. The helical wheel projection reveals that one face of the helix is highly conserved. It consists of three leucines, an alanine, a glycine, and three more leucines. Only in two cases is one of the leucines replaced by an isoleucine. The neighboring face of the helix contains a conserved hydrophobic and conserved charged amino acids, two identical arginines, an acidic residue, and a less conserved basic residue. The other parts of the helix are less well conserved.

As described recently, screens for the temperature-sensitive vti1 mutants in yeast led to the identification of two different classes of mutants (25). The mutants vti1-1 and vti1-2 exhibit a block in transport of the vacuolar hydrolase CPY from the late Golgi to the prevacuolar compartment at the restrictive temperature. The mutant vti1-11 has a temperature-sensitive growth defect and accumulates secretory proteins in the ER and early Golgi compartment, in addition to a block in Golgi to prevacuolar traffic. To determine which parts of Vti1p are involved in these functions the vti1 mutant alleles were sequenced. vti1-1 contains the amino acid exchanges E145K and G148R. vti1-2 has the mutations S130P and I151T. 8 amino acid exchanges were identified in vti1-11 (Y8R, K20R, H40R, N61S, K73R, Q84R, E145G, and L155F). The construction of a hybrid protein encoded by the plasmid pFvM108 revealed that yeast cells carrying a protein with only the amino acid exchanges E145G and L155F exhibited phenotypes identical to the original vti1-11 mutant (data not shown). Therefore E145G and L155F are responsible for the trafficking defect observed for vti1-11.

In our ongoing analysis of yeast Vti1p function we analyzed a new VTI1 allele, vti1-12. The fate of newly synthesized CPY was monitored in vti1-12 cells incubated at 22 °C or for 15 min at 37 °C by pulse-chase labeling followed by CPY immunoprecipitation. Even at 22 °C vti1-12 cells accumulated a significant proportion of CPY in the ER and early Golgi forms (p1CPY) and the late Golgi form p2CPY intracellularly (42). Hardly any vacuolar localized mature CPY (mCPY) was present (Fig. 3, left panel, E). These cells also secreted p2CPY (Fig. 3, left panel, E). At 37 °C vti1-12 cells exhibited a severe growth defect and accumulated an even higher proportion of CPY in the ER and
expressed under the VTI1 promoter were transformed into the multicopy yeast expression plasmid pVT100-U through PCR amplification (codons 62–232) were amplified and the coding region for the shorter version of hVti1 identified.

The coding regions for the complete hVti1 (codons 1–232) and of hVti1 further, two expression plasmids were constructed.

As mentioned above, hVti1 was identified as a suppressor protein that allowed growth of wild type cells (squares) and vti1Δ cells expressing hVti1 amino acids 62–232 (diamonds), or complete hVti1 (1–232, circles). The doubling times for vti1Δ cells expressing hVti1 (62–232) and hVti1 (1–232) were essentially identical (3 h) and only slightly longer than the doubling time of wild type cells (2 h, 10 min). Cells were grown in rich medium at 30 °C in logarithmic phase. Cell density was measured by optical density at 600 nm.

Restoration of CPY Transport by Expression of hVti1—hVti1 (62–232) was expressed in the temperature-sensitive mutants vti1-1 and vti1-11 to assay its effect on the two trafficking steps that have been shown to require Vti1p. At the restrictive temperature, vti1-1 cells secreted and accumulated intracellularly as p1CPY, and some late Golgi p2CPY, which got past this first block, was secreted and did not reach the vacuole. Expression of hVti1 (62–232) in vti1-11 cells restored transport of CPY to the vacuole.

To determine if hVti1 acts as a bypass suppressor or together with the mutant Vti1p, hVti1 was expressed in vti1Δ cells. In vti1Δ cells expression of hVti1 (62–232) allowed a significant fraction of CPY to reach the vacuole (Fig. 5, bottom left panel), and some CPY was secreted. These data indicate that hVti1 (62–232) can function in both Golgi to prevacuolar transport and in traffic to the cis-Golgi in the complete absence of yeast Vti1p.

Expression of the complete hVti1 (1–232) in vti1Δ cells (Fig. 5, bottom right panel) gave results similar to expression of the shorter version hVti1 (62–232). This suggests that the N-terminal 61 amino acids of hVti1 are not needed for function in yeast. Maturation of CPY was slower in vti1Δ cells than in vti1-ts cells expressing hVti1 and a proteolytic intermediate was observed at earlier time points (data not shown). Rather than reflecting slower kinetics of vacuolar delivery this likely indicates that the vacuole contains lower amounts of proteases in vti1Δ cells expressing hVti1 than in wild type cells.

Suppression of the Invertase Sorting Defect by hVti1—Trafﬁcking through the secretory pathway was further studied using the secreted protein invertase (Fig. 6). Cells were shifted to 37 °C and newly synthesized invertase was radiolabeled during the pulse and immunoprecipitated immediately or after a 30-min chase period. Invertase received extensive Asn-linked carbohydrates in the ER during the pulse period (Fig. 6, lane 9) and was converted to a slow migrating, heterogeneous glycoprotein in the Golgi complex, and then rapidly secreted in wild-type cells (Fig. 6, lanes 10 and 12) (33). In vti1-11 cells the core glycosylated ER form of invertase accu-
FIG. 5. Suppression of the CPY sorting defect by hVti1 expression. CPY expression was followed in vti1-1 cells (top left panel) and vti1-11 cells (top right panel) in the absence (−, left) or presence (+, right) of hVti1 (amino acids 62–232) at 37 °C after a 15-min preincubation. hVti1 suppressed the block of Golgi to vacuolar transport in vti1-1 cells, demonstrated by the presence of intracellular mCPY. hVti1 also suppressed the cis-Golgi traffic block in vti1-11 cells. In the absence of Vti1p (vti1Δ, bottom panels) expression of hVti1 (amino acids 62–232) or the complete hVti1 (1–232) allowed sorting of CPY to the vacuole with similar efficiency. Pulse-chase labeling was performed at 30 °C in vti1Δ strains.

FIG. 6. Suppression of the invertase secretion defect by hVti1 expression. Invertase expression was followed in vti1-1 cells (62–232), and wild type cells were grown at 24 °C and preincubated (15 min) and labeled at 37 °C. Invertase immunoprecipitations from intracellular (I) or extracellular (E) fractions were performed directly after the 7-min pulse or after a 30-min chase. In vti1-1 cells the ER form of invertase accumulated and a severely under glycosylated form was secreted (left panel). Expression of hVti1 in vti1-11 cells led to the accumulation and secretion of mature and underglycosylated invertase. The ER form of invertase was absent (middle panel). In wild type cells, mature invertase was secreted (right panel).

mulated intracellularly (Fig. 6, lane 3, Inv. ER) and a severely underglycosylated form was secreted after a 30-min chase at the restrictive temperature (Fig. 6, lane 4) (25). No mature invertase was formed in vti1-11 cells shifted to the high temperature. These data indicate that ER to Golgi membrane traffic is drastically slowed down and that the Golgi apparatus has lost the ability to glycosylate invertase normally in vti1-11 cells at restrictive temperature. In vti1-11 cells expressing hVti1 (62–232) the core glycosylated ER form of invertase, which was found after the pulse-labeling period (Fig. 6, lane 5), was absent after the chase (lane 7). Instead, mature and underglycosylated invertase were found both within the cell and in the secreted fraction (lanes 7 and 8). These data indicate that expression of hVti1 suppressed the block in traffic to the cis-Golgi compartment, which was observed in vti1-11 cells. A portion of the invertase was not secreted and/or remained underglycosylated after the 30-min chase period, suggesting that Golgi function was only partially restored.

**DISCUSSION**

**Identification of the Human Vti1 Homolog**—A human homolog (hVti1) of the yeast Vti1p was identified by functional complementation. A mouse homolog, mVti1, which is 93% identical to Vti1 over the full length of the protein, was assembled from data base EST sequences. The homology did not extend upstream of the putative initiating methionine, indicating that the proteins are in fact 232 amino acids long. In addition, two clones amplified by PCR as well as a human EST (AA056932) found in the data base encode hVti1 starting from methionine 62 but contain three different sequences upstream. These clones could represent cloning artifacts. However, it is clear that both the full length and the short version are functional in yeast. Therefore, it is also possible that the different 5' ends of the mRNA could be derived through alternative splicing and that both proteins could exist in humans. This would require that the spliced RNAs are of similar length because only one mRNA band was identified in Northern blots of RNA from human tissues.

Two mouse proteins, mVti1 and mVti1b, were assembled from the EST database and they exhibit a similar homology to yeast Vti1p (27–35%). Surprisingly, mVti1 and mVti1b share only 31% amino acid identity. Redundant proteins are common in mammals, but are usually much more similar to each other. Two different mammalian Sec22p homologs have been identified. Although from related species (rat and hamster), rSec22 and ERS-24 share only 35% amino acid identity (9, 10). It remains unclear whether these proteins function in the same or in different transport steps. The tagged and overexpressed rSec22 protein localizes to the ER, whereas ERS-24 localizes to the ER and Golgi. In vitro ERS-24 was found in a complex with Sed5p. The amino acid sequences of the redundant v-SNAREs synaptobrevin1/VAMP1 and synaptobrevin2/VAMP2 are 77% identical (43, 44). In contrast, cellubrevin, which acts in a similar but distinct membrane traffic step, has 59% amino acid identity with the other mammalian synaptobrevins (45). It therefore seems more likely that mVti1 and mVti1b function in different trafficking steps.

**Implication for the Structure of v-SNAREs**—In an effort to determine which domains of v-SNAREs and t-SNAREs interact, several groups have characterized different SNARE fragments in *in vitro* binding assays. Syntaxin 1 interacts through the putative coiled helix 3 (amino acids 190–240) with both synaptobrevin and SNAP-25 (46, 47). SNAP-25 binds to syntaxin through the N-terminal predicted coiled coil region (amino acids 1–100). SNAP-25 requires its N- and C-terminal coiled coil regions for binding to synaptobrevin. These data suggest that t-SNAREs interact via coiled coil domains with v-SNAREs. Isolated SNAP-25 has a very low a-helical content, but in a SNAP-25 syntaxin complex the a-helicity is increased dramatically (36). The related yeast SNAREs behave in a similar way. The isolated v-SNARE Snc1p and the t-SNARE Sec9p are largely unstructured (35). The formation of a-helices is induced in a complex of Snc1p, Sec9p and Sso1p and thermal stability is increased. These data indicate that SNAREs exist in different conformations and that coiled coil structures are induced by complex formation. The transition between unfolded and folded states may play an important role in docking and fusion. *In vitro* binding assays have not been particularly successful to narrow down the interacting domains of v-SNAREs. Synaptobrevin2 requires most of the cytosolic domain (amino acids 27–96) for its interaction with syntaxin (48). In a functional assay, amino acids 31–89 of synaptobrevin2 were required to restore exocytosis (49).

Using sequence analysis of temperature-sensitive vti1 mutants we have been able to narrow down the functional domain of Vti1p. We analyzed two mutants defective in Golgi to pre-vacuolar transport (mediated by the prevacuolar t-SNAREs Pep12p), and two mutants with additional defects in trafficking to the cis-Golgi, which involves the cis-Golgi t-SNARE Sed5p.
Amino acid exchanges in both classes of mutants cluster in a very narrow region between amino acid residues 130 and 158. This area is predicted to be α-helical and to fold into a coiled-coil structure with low probability. It is immediately adjacent to the domain that is predicted to form a coiled-coil structure with higher probability (amino acids 159–188). In analogy to Snclp, Sec9p and SNAP-25, folding may require the presence of a t-SNARE.

Functional Complementation—hVti1 was capable of replacing Vti1p in both Golgi to prevacuolar transport and traffic to the cis-Golgi compartment. The first 61 amino acid of hVti1 protein’s structure and key functional residues must be conserved. The most conserved feature among the different Vti1-mammalian Vti1 homologs and their ability to suppress the growth defect of vti1-ts mutants are in evolution-conserved residues in a domain that is predicted to form a coiled coil structure with α-helix between amino acid residues 43 and 69. hVti1 contains an additional amphipathic domain (amino acid residues 77–98), and these domains may be functionally redundant. The first 61 amino acid of hVti1 is quite surprising given the low degree of amino acid identity between amino acids 134 and 181. All the amino acid exchanges in both classes of mutants cluster in a very narrow region between amino acid residues 130 and 158. This means that hVti1 does not need most of the conserved predicted amphipathic α-helix between amino acid residues 43 and 69. hVti1 contains an additional amphipathic domain (amino acid residues 77–98), and these domains may be functionally redundant. The ability of hVti1 to functionally complement in yeast is quite surprising given the low degree of amino acid identity between the human and the yeast proteins (29%). Clearly, the protein’s structure and key functional residues must be conserved. The most conserved feature among the different Vti1-like proteins is a hydrophobic face in a predicted α-helix between amino acids 134 and 181. All the amino acid exchanges identified in the mutant yeast Vti1p proteins are in evolutionary-conserved residues in this region and on the same side of the predicted α-helix. Therefore, it seems very likely that this hydrophobic face, which is lined by charged residues, represents the part of Vti1p that interacts with its effector proteins, possibly with both Pep12p and Sed5p.

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Note Added in Proof—After this paper was in press, Lupashin et al. (Lupashin, V. V., Pokrovskaya, I. D., McNew, J. A., and Waters, M. G. (1997) Mol. Biol. Cell 8, 2659–2676) reported the sequences of mammalian Vti1 homologs and their ability to suppress the growth defect of vti1-ts cells.
A Human Homolog Can Functionally Replace the Yeast Vesicle-associated SNARE Vti1p in Two Vesicle Transport Pathways
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