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mVps24p functions in EGF receptor sorting/trafficking from the early endosome

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Abstract

Yeast Vps24p (**v**acuolar **p**rotein **s**orting) is part of a protein complex suggested to function in sorting/trafficking during endocytosis. We have characterized a mammalian homolog of the yeast protein, mVps24p, and examined its role in epidermal growth factor receptor trafficking. Endogenous mVps24p was distributed in both cytosol and in puncta and partially colocalized with markers for the trans-Golgi network. Adventitious expression of hrs or a mVps4p mutant deficient in ATPase activity caused a redistribution of both mVps24p and the M6PR to the resultant clustered/enlarged early endosomes. Expression of an mVps24p N-terminal fragment, that interacts with phosphatidylinositol 3,5-bisphosphate but not with mVps4p, produces enlarged early endosomes. More importantly, the mVps24p N-terminal fragment resulted in not only enhanced recycling, but also decreased degradation of the EGF receptor. These findings are consistent with a model in which mVps24p has a role in trafficking from the early endosome.

Keywords: Hrs; Vacuole; Endosome; Mucolipidosis type II; Mannose 6-phosphate receptor; EGF; Vps4; Receptor

Introduction

The endosomal system includes early, late, and recycling endosomes as well as various types of transport vesicles. Together, they function in the sorting and delivery of endogenous and endocytosed molecules to specific destinations within the cell [1,2]. Early endosomes are the first organellar recipient of endocytosed material. They are also major branch points in the endocytic pathway where molecules destined for recycling are separated from those that will ultimately be degraded in the lysosome [1,2].

Hepatocyte growth factor-**r**egulated tyrosine kinase **s**ubstrate (Hrs) was isolated by virtue of its growth factor-induced phosphorylation [3], and its binding to the SNARE protein SNAP-25 [4]. Hrs is clustered in domains on the cytoplasmic surface of the limiting membrane of endosomes [5,6]. Enlarged early endosomes form in tissues of mouse Hrs knockout embryos and overexpression of myc-tagged Hrs in cultured cells induces a similar phenotype [5,6]. Hrs plays a role in endosome fusion as well as to recruit proteins to the endosomal membrane that may function in cargo sorting [7,8]. The m*VPS4* gene encodes a human homolog of a yeast protein, Vps4p [9], required for endocytic sorting. Mutations in m*VPS4* that prevent ATP binding or hydrolysis induce the formation of enlarged vacuoles that contain both endosomal and lysosomal markers [9].

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Here we describe the mammalian class E protein, mVps24p, its endogenous localization, and the effect of expressing the N-terminal domain of mVps24p (N-mVps24p) containing a lipid-binding domain. Expression of N-mVps24p results in an increase in recycling and a decrease in degradation of the EGF receptor, suggesting a role for mVps24p in a sorting event at the early endosome.

Materials and methods

Cell lines and transient transfections

All products for cell culture were purchased from Gibco/BRL. Primary human fibroblast cell lines GM00038C (9-year-old female control) and GM03066D (23 fetal week female MLII) were purchased from Coriell Cell Repositories (Camden, NJ). Fibroblasts were grown in DMEM supplemented with 20% FBS and 100 U/ml penicillin and 100 mg/ml streptomycin. Fibroblasts were transfected by electroporation with a BTX Electro Cell Manipulator 600 at 129 W, 1500 μF, 255 V. In each case, between 1×10^6 and 3×10^6 cells were isolated following trypsinization, washed in Opti-MEM®I (Gibco/BRL), and electroporated in cuvettes (4-mm gap, BTX model 640) in 500 ml Opti-MEM with 10 mg endotoxin-free plasmid DNA. Transfected cells were plated onto cover slips and fixed in 4% PFA (30 min) 2–3 days after electroporation. HeLa cells were plated and transfected (Polyfect, Qiagen) 24 h prior to fixation with 4% PFA (15 min) and washing with PBS.

Antibodies and immunocytochemistry

Polyclonal rabbit antisera were raised against a peptide (Stanford PAN Facility) corresponding to the first 15 amino acids of mVps24p (MGLFGKTQEKPPKEL) coupled to keyhole limpet hemocyanin (Pierce). Anti-mVps24p antibodies were purified on columns of GST/mVps24p bound to glutathione Sepharose (Amersham Pharmacia Biotech AB) using dimethylpimelimidate (Sigma).

Mouse primary antibodies to EEA1 were purchased from BD Transduction Labs. Antibodies to the cation-independent mannose 6-phosphate receptor (M6PR) were purchased from Research Diagnostics, Inc. Mouse primary antibodies to c-myc were purchased from the Developmental Studies Hybridoma Bank. Secondary antibodies were purchased from Jackson ImmunoResearch Laboratories. Fixed fibroblasts were permeabilized for 5 min in 1% saponin in PBS for all primary antibodies. Cells were either blocked for 20 min in 2% goat serum in PBS or 2% goat serum with 1% saponin prior to incubation (60 min or overnight) with primary antibody in blocking buffer. Cover slips were washed seven times in PBS prior to 30-min incubation with secondary antibody. Following additional washing, cover slips were mounted in antifade medium (paraphenylamine diamine).

Fluorescence microscopy and image analysis

Cells were viewed with a 100× oil objective lens on a Zeiss Axioplan microscope and images were captured with a digital camera [either an AxioCam digital camera and the AxioVision 2.0 imaging system (Zeiss), or a Hammatsu Orca and Metamorph Software (Universal Imaging)]. Confocal images were processed on a Noran Oz deconvolution microscope at The Johns Hopkins University School of Medicine Microscope Facility.

Sequence analysis and generation of recombinant proteins

Using yeast Vps24p sequence (GenBankNP_012883) as a query, we performed BLAST searches and compiled human ESTs predicted to encode a human homolog (mVps24p). Three unique sequences for human homologs of Vps24p were compiled. We chose the one with the highest amino acid identity to Vps24p (30%) for study and named this protein mVps24p. The chromosomal region corresponding to m*VPS24* gene was scanned for potential linked diseases in the OMIM, PubMed, and Ensembl databases. The m*VPS24* cDNA was cloned from human heart cDNA (OriGene) by polymerase chain reaction using oligonucleotides 5′ GCT CTA GAC ATG GGG CTG TTT GGA AAG ACC 3′ and 5′ CCC GTC GAC CTA GCT GCG GAG TGT GGC CAG 3′ and ligated into pGEX-KG and pEGFP (Clontech) vectors. The nr protein and DNA databases at GenBank were further searched for sequences from other species homologous to Vps24p. The predicted amino acid sequences were analyzed using the Genetics Computer Group peptidesort and PILEUP programs with default penalty values (Accelerys, Inc.) and the COILS program (http://www.ch.embnet.org) [10]. The Phylogeny Analysis Using Parsimony (PAUP) program (Sinauer Associates, Inc.) was used to generate a phylogenetic tree using a maximum parsimony criterion. GST/mVps24p fusion protein was expressed in DH10B *E. coli* cells and purified as previously described [11]. Constructs encoding h*VPS4* were a generous gift of Philip Woodman and the N-mVps24p construct was a kind gift of Paul Whitley.

Endocytic trafficking

For ^{125}I-EGF trafficking, either untransfected HeLa cells or HeLa cells that had been transfected with pEGFP-N-mVps24, or pEGFP vector, were starved (medium A, 1% BSA in DMEM, 60 min at 37°C), labeled with ^{125}I-EGF (1 ng/ml) for 30 min at 37°C, then rinsed with cold medium A twice, acidic solution (0.15 M NaCl, 0.1 M glycine pH 3.0) twice, and once again with medium A. Cells were chased with medium A for the indicated times. The media and cells were collected at each time point. Cells were harvested by scraping with 1 M NaOH. Proteins in the media were precipitated (20%

TCA) and cells (internalized EGF) and media pellet (recycled EGF) were counted in a γ-counter. Each data point was collected in duplicate. Recycled ^{125}I-EGF was expressed as the ratio of recycled ^{125}I-EGF versus internalized ^{125}I-EGF. The data were corrected for non-specific cell-associated ^{125}I-EGF (<10%) as determined in parallel experiments in which an excess (200 μg/ml) of unlabeled EGF present during labeling. Kinetic parameters were obtained by fitting data with a linear regression over time. Bar graphs present mean slopes obtained by regression analysis. Differences between rates were analyzed using ANOVA with post hoc Bonferroni test. Transfection efficiency, based on transfection of cultures with CFP-EGFR, are approximately 70%. Since the efficiencies are rather high, although not complete, the effects we observe in kinetic measurements are at the very least conservative estimates. The data are statistically significant, suggesting that the experimental variation that could result from wavering efficiency is not excessive. The localization of the EGFR was examined after incubation of cells that had been transfected 24 h prior with either N-mVps24-GFP or GFP. Cells were incubated with EGF (100 ng/ml) for 10 min and fixed (4% PFA) either 0 or 120 min later. EGFR localization was determined after incubation with primary EGFR antisera and secondary antisera conjugated to Alexa 594. Cells were viewed with a 100× oil objective lens on a Zeiss Axioplan microscope and images were captured with a digital camera [Hammatsu Orca and Metamorph Software (Universal Imaging)].

To examine degradation of the EGF receptor, HeLa cells were transfected with pGFP-N-mVps24p, the GFP vector, GFP-hrs or mock-transfected and transfected again with the same plasmid DNA 24 h later. Cells were starved in 1% BSA DMEM medium for 1 h, then induced to undergo receptor-mediated endocytosis with 100 ng/ml EGF in the presence of 25 μg/ml cycloheximide for 0 or 30 min at 37°C. Cells were washed with PBS, lysed in buffer, and proteins were separated using SDS-PAGE. The amount of EGFR in the lysates was determined by immunoblotting with anti-EGFR and quantitated using NIH Image (ver 1.62).

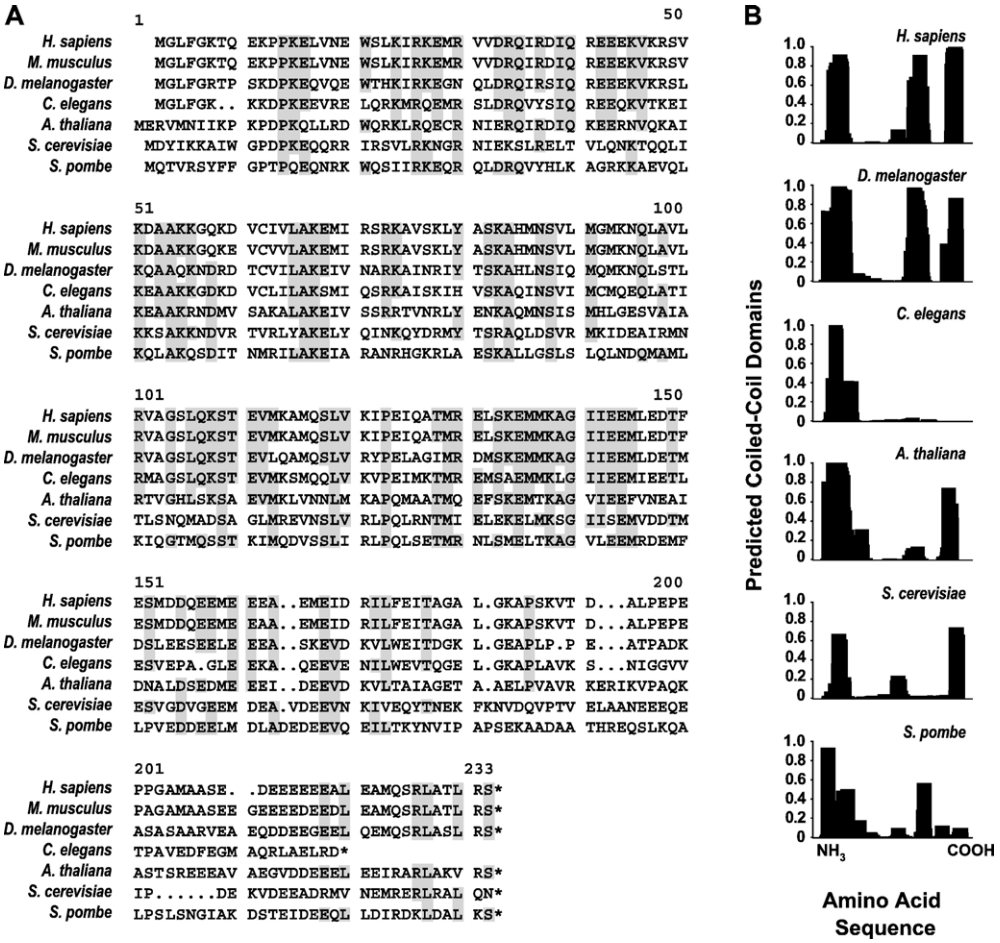

Fig. 1. Sequence analysis of Vps24p homologs. (A) Multiple sequence alignment for homologs of Vps24p including *Homo sapiens* (Hs), *Mus musculus* (Mm), *Drosophila melanogaster* (Dm), *Caenorhabditis elegans* (Ce), *Arabidopsis thaliana* (At), *S. cerevisiae* (Sc) and *Schizosaccharomyces pombe* (Sp). An asterisk signifies a stop codon. Amino acids shared by six of the seven homologs are shaded. (B) Coils outputs for the homologs of Vps24p. The predicted amino acid sequence spans the *x*-axis. The probability of coil formation is indicated on the *y*-axis. The black bars represent predicted coiled-coil regions.

Results

Identification and cellular localization of mVps24p

Mammalian homologs of yeast class E proteins have been identified and each has a role in trafficking through endosomes [5,8,12–17]. To better understand the role of mVps24p in endosomal trafficking, we cloned a cDNA encoding mVps24p, a potential human ortholog of the *S. cerevisiae* class E gene *VPS24*. During preparation of this manuscript, Whitley et al. [18] reported cloning m*VPS24*. This sequence was also described in GenBank as CGI-149 (accession NP_057163), one of over 150 putative human proteins identified by comparative proteomics between the *Caenorhabditis elegans* proteome and human EST nucleotide databases[19]. *VPS24* and m*VPS24* are predicted to encode 25-kDa proteins with 30% amino acid identity between them. The relationships among proteins predicted from sequences corresponding to the *VPS24* gene from seven eukaryotic species are displayed as a multiple amino acid sequence alignment (Fig. 1A). The sequence of *S. cerevisiae* Vps24p has an unequal charge distribution [12], and each predicted Vps24p homolog shares this feature, with a high isoelectric point (between 10.7 and 11.6) for the amino-terminal half of the protein and a low isoelectric point (between 3.9 and 4.3) for the carboxy-terminal half. All of the Vps24p homologs analyzed are predicted to have coiled-coil domains (Fig. 1B). The m*VPS24* gene is on human chromosome 2p24, a region that does not appear to be linked to any genetic disorders, although losses and amplifications of 2p24 are found in several different cancers (e.g., Refs. [20,21]).

Antisera was raised against a peptide within the amino terminus of mVps24p. It detected the 28-kDa recombinant mVps24p cleaved from a GST fusion protein with thrombin (Fig. 2A, lane 1). This antisera also recognized an endogenous protein of approximately 28 kDa and weakly reacted with a 37-kDa protein from human fibroblast lysate (Fig. 2A, lane 2). These antibodies also detected EGFP/mVps24p at the expected size of about 60 kDa (Fig. 2A, lane 3). Antisera preincubated with excess peptide antigen did not bind to any proteins on a replicate blot, confirming the specificity of the antisera (Fig. 2B). Immunocytochemistry on normal human fibroblasts detected small, granular structures that were diffusely localized throughout the cell, in addition to larger punctate or tubular structures in the nuclear and perinuclear regions (Fig. 2C). Preincubating anti-mVps24p antiserum with the peptide antigen also completely blocked detection of mVps24p (Fig. 2D).

The endogenous localization of mVps24p was examined in HeLa cells and primary human fibroblasts. This revealed a similar punctate expression pattern with slightly increased numbers of puncta observed in a perinuclear localization (Figs. 3A, D, G, J). The trans-Golgi Network markers, M6PR [22] (Figs. 3E, F, K, L), and syntaxin 6 [23] (Figs. 3H, I) but not the early endosomal marker EEA1 (Figs. 3B, C) partially

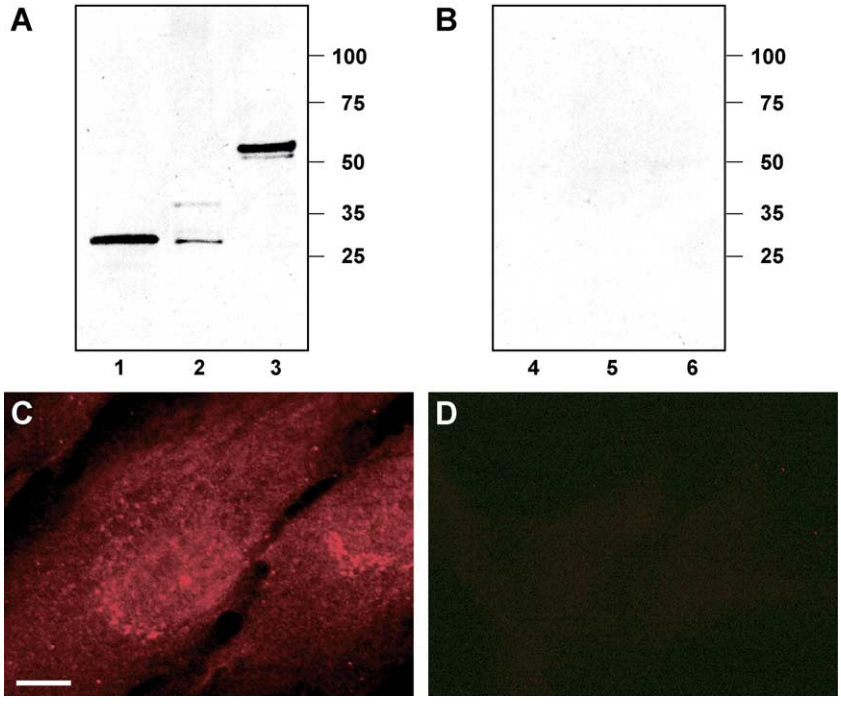

Fig. 2. Antibodies to mVps24p specifically recognize a 28-kDa protein that localizes to punctate structures. (A) Western blot using anti-mVps24p. Lane 1, 5 ng recombinant mVps24p, cleaved from a GST fusion protein with thrombin. Lane 2, 40 μg control fibroblast lysate. Lane 3, 10 μg lysate from fibroblasts transfected with EGFP/mVps24p. (B) Replicate Western blot (in A) using anti-mVps24p antiserum after a preincubation for 30 min with the peptide antigen. (C) Immunocytochemistry using anti-mVps24p on wild-type human fibroblasts (95 ms exposure). (D) Anti-mVps24p signal was blocked with peptide antigen (100-ms exposure). The white bar in C indicates 10 μm in C–D.

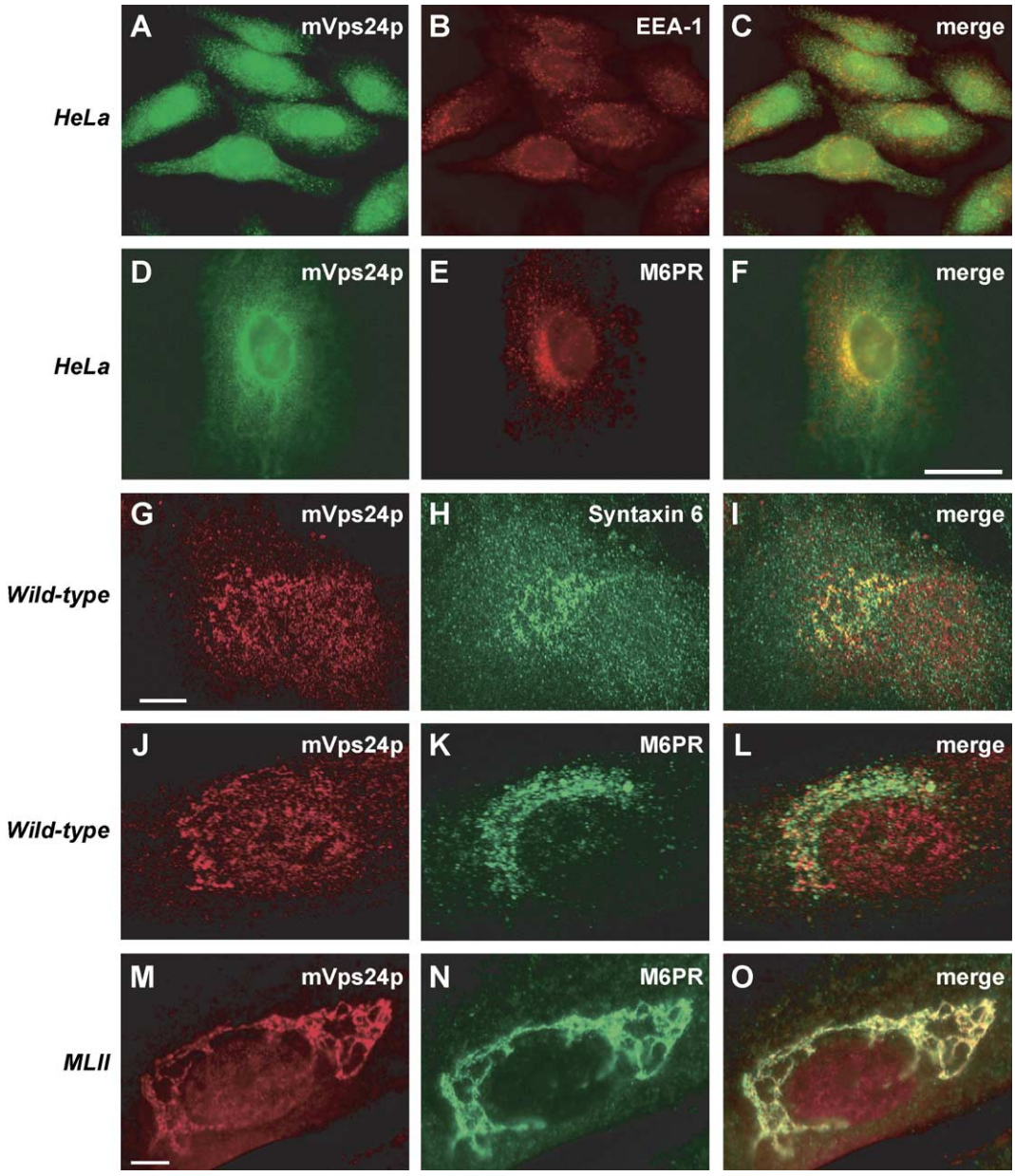

Fig. 3. mVps24p partially localizes to the TGN in HeLa cells and fibroblasts. Images of HeLa cells labeled with anti-mVps24p (A, green) and anti-EEA-1 (B, red; C, merged image). Labeling with anti-mVps24p (D, green) and anti-M6PR (E, red; F, merged image). The white bar in F indicates 15 μm in A–F. Confocal image of control human fibroblasts co-stained with anti-mVps24p (G, red) and anti-syntaxin 6 (H, green; I, merged image). Confocal image of control cells co-stained with anti-mVPS24 (J, red) and anti-M6PR (K, green; L, merged image). Fluorescence microscopy image of MLII fibroblasts co-stained with anti-mVPS24 (M, red) and anti-M6PR (N, green; O, merged image). The white bar in G indicates 10 μm in G–L. The white bar in M indicates 10 μm in M–O.

overlapped with the distribution of mVps24. These data suggest that the steady state distribution of mVps24p includes membranes of the trans-Golgi network.

A defect in *N*-acetylglucosamine-1-phosphotransferase, the enzyme that synthesizes the mannose 6-phosphate signal on soluble lysosomal enzymes, causes the lysosomal storage disorder Mucolipidosis Type II (MLII) [24,25]. Mannose-6-phosphate is required for M6PR-mediated transport of lysosomal enzymes from the TGN to endosomes [22]. In MLII fibroblasts, the steady state distribution of the M6PR shifts from perinuclear late endosomes to the TGN, which has an aberrantly reticularized structure in these cells[26]. Thus, MLII fibroblasts have distinct alterations both in protein distribution and in TGN architecture, making them a useful system to analyze putative trafficking factors.

In MLII cells, mVps24p was not visualized as perinuclear puncta or cytoplasmic granules but instead localized to distinct reticular structures (Figs. 3M–O). The localization of the M6PR also on this reticular structure indicated that the majority of mVps24p was on the TGN in MLII fibroblasts. The localization of mVps24p was not altered in fibroblasts from patients with Mucolipidosis Type IV or

Niemann–Pick Type C (data not shown). Defects in traffic from late endosomes and lysosomes cause these lysosomal storage disorders, respectively (for reviews, see Refs. [27,28]).

Inhibition of lysosomal trafficking alters the steady state distribution of mVps24p

The yeast and human class E AAA-ATPases, scVps4p and mVps4p, cause the dissociation or uncoating of class E protein complexes from endosomal membranes [9,12,15,29]. Expression of ATPase-defective mVps4p induces formation of greatly enlarged organelles similar to the yeast class E compartment since they accumulate endosomal and lysosomal resident proteins as well as endocytosed markers [9]. Nucleotide binding regulates the association of *S. cerevisiae* Vps4p with yeast endosomal membranes in vivo and this in turn regulates the membrane association of some other class E proteins, including Vps24p [12]. Cells transfected with EGFP/hVps4EQ contained aberrant, enlarged structures (Fig. 4A), consistent with previous reports [9,15]. The distribution of mVps24p shifted to the surface of a subset of EGFP/hVps4EQ-induced structures (Fig. 4B), indicating a function for mVps4p in regulating the endosomal membrane association of mVps24p. Expression of wild-type mVps4p did not induce redistribution of mVps24p (data not shown). EGFP/hVps4EQ also induced the redistribution of lysosomal integral membrane protein 1 (LIMP1) (Fig. 4C). LIMP1 (also called CD63/LAMP-3) normally localizes specifically to late endosomes and lysosomes [30]. A merged image of EGFP/hVps4EQ, mVps24, and LIMP1 signal revealed extensive colocalization in transfected cells but essentially no colocalization in nontransfected cells (Fig. 4D). These results were consistent with a model in which mVps24p associates with other class E proteins on endosomes and the ATPase function of mVps4p is required to disassemble the complex. It is interesting to note that the M6PR localizes to the mVps4EQ-induced vacuoles (data not shown, and Ref. [15]), indicating that mVps4p, like *S. cerevisiae* class E proteins, may also be required for the endosomal exit of membrane proteins that cycle out of endosomes.

Overexpression of hrs blocks endosomal sorting/trafficking at the level of early endosomes and results in endosomal aggregation and enlargement [6,31]. This effect is likely the result of mimicking a null phenotype by titrating hrs-interacting factors from their binding/functional sites. Overexpression of hrs altered the localization of mVps24p, redistributing the protein to the enlarged hrs-containing vacuoles (Figs. 4F, H). Interestingly, hrs expression also altered the localization of M6PR resulting in its localization on hrs-positive vacuoles (Figs. 4G, H). These data suggest that hrs either recruits mVps24p to aberrant structures directly, indirectly, or by virtue of blocking trafficking out of these structures. We have examined whether hrs and mVps24p directly interact using immunoprecipitation and recombinant protein binding and have not detected a direct interaction (data not shown). As a result of hrs expression, mVps24p may be either recruited

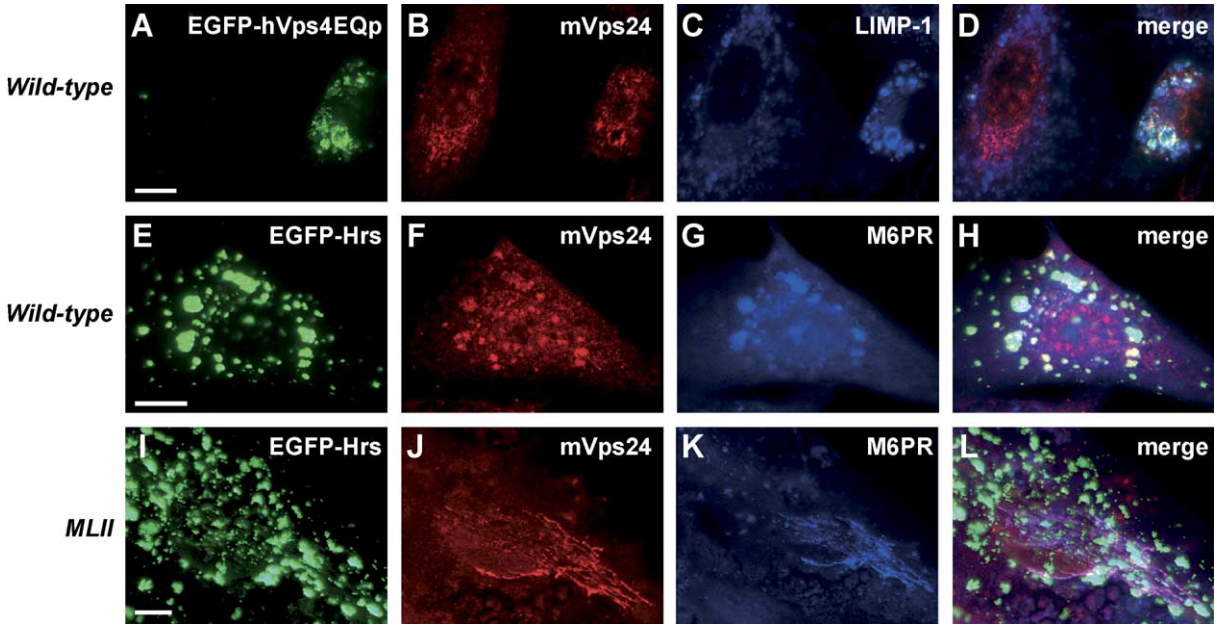

Fig. 4. Disrupted trafficking through endosomes induces redistribution of mVPS24p. Expression of EGFP/hVPS4EQ (A, green) alters the distribution of mVps24p (B, red) and LIMP-1 (C, blue; D, merged image). In control cells, high levels of expressed EGFP/Hrs (E, green) altered the distribution of mVps24p (F, red) and the M6PR (G, blue) such that mVps24p and the M6PR colocalized on Hrs induced organelles (H, merged image). In MLII cells, high levels of Hrs expression (I, green) induced the formation of enlarged endosomes with irregular borders; however, mVps24p (J, red) and the M6PR (K, blue) did not shift from the TGN to these enlarged early endosomes in MLII cells (H, merged image, fuchsia indicates colocalization of mVps24p and the M6PR). The white bar in A indicates 10 μm in A–L.

to early endosomes by virtue of binding to another molecule retained on these aberrant endosomal membranes or it may be mislocalized due to the hrs-induced trafficking defect. However, in MLII cells, hrs did not induce a redistribution of mVps24p or the M6PR; instead, they colocalized on the reticulated TGN, characteristic of MLII cells (Fig. 4). Thus, in the absence of transport from the TGN to endosomes as occurs in MLII [24,25], a block in trafficking through early endosomes does not cause a redistribution of mVps24p, suggesting that its endosomal association is likely determined by a receptor as has been suggested for the yeast protein [32].

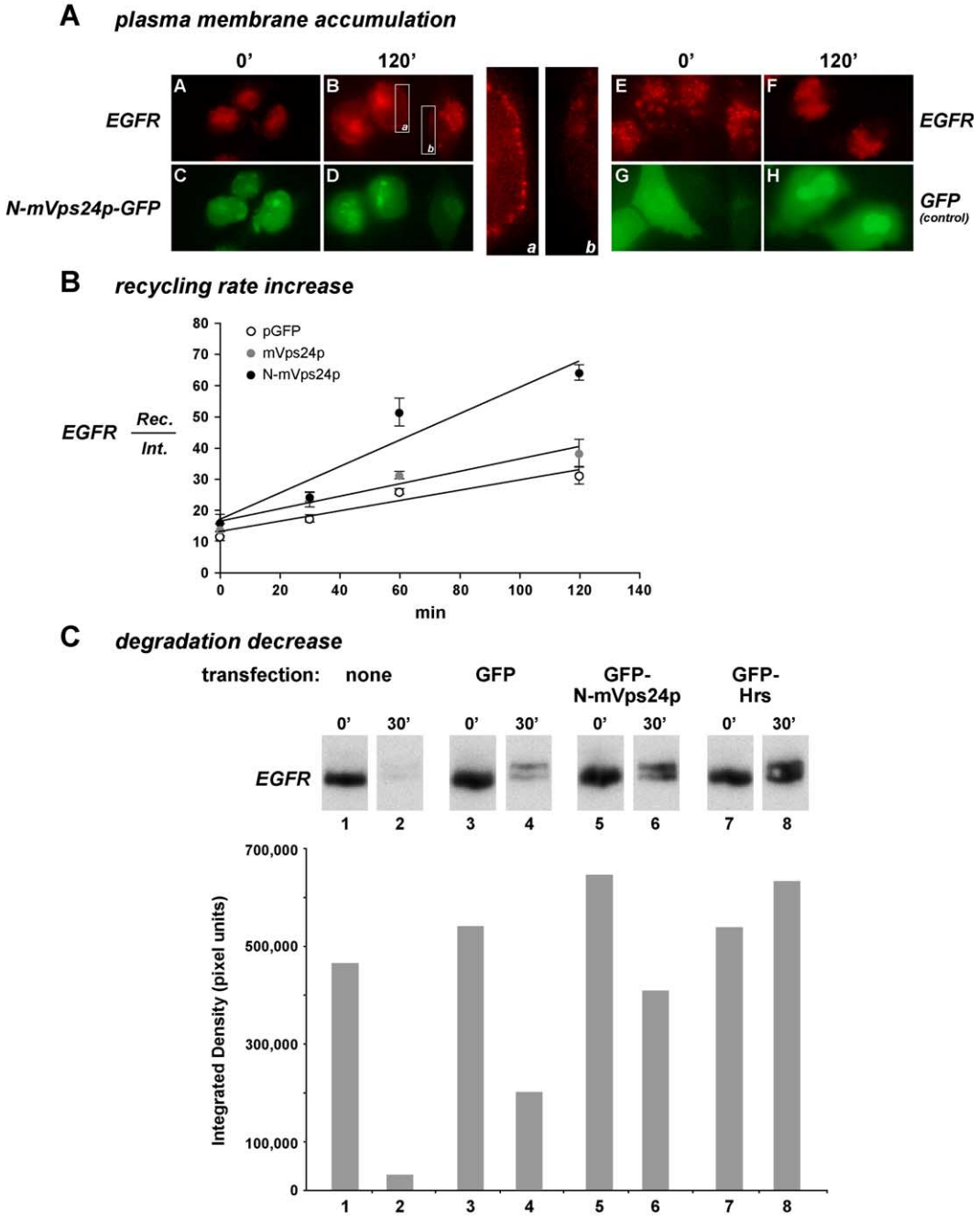

Fig. 5. Expression of N-mVps24p increases recycling and decreases degradation of EGF receptors. (Panel A) HeLa cells expressing either N-mVps24-GFP (parts C, D) or GFP (parts G, H) were serum starved and incubated for 30 min with EGF (100ng/ml). After an acid wash to remove cell surface EGF, cells were either fixed (0 time) or chased (120 min). The localization of N-mVps24p is both punctuated and cytosolic, and 120 min after EGF stimulation, immunolabeling for the EGFR revealed that it can be observed in small punctate spots on the plasma membrane (parts B, a) whereas in cells transfected with GFP, the EGFR has been largely degraded (parts F, b). (Panel B) The kinetics of EGFR recycling were determined in cells expressing GFP, mVps24p, or N-mVps24p (B), measured by examining the distribution of ^{125}I-EGF. The rate of recycling (left side) in cells expressing N-mVps24p was approximately double that of either the GFP- or mVps24p-expressing cells (B). (Panel C) The rate of degradation of the EGFR was slowed in cells expressing N-mVps24p (lanes 5, 6 vs. 1, 2).

N-mVps24p expression increases recycling of EGF receptors

To study a possible role of mVps24p on early endosomal traffic, we expressed the N-terminal fragment of mVps24p. This fragment of mVps24p interacts with phosphatidylinositol 3,5-bisphosphate [18] and accumulates on membranes presumably due to its lack of disassociation that is normally driven by the ATPase activity of Vps4p when bound to mVps24 [18]. We examined the kinetics of EGF receptor trafficking in cells expressing N-mVps24p and control cells. Cells expressing N-mVps24p increased their rate of EGF receptor recycling compared to controls (Fig. 5, $P < 0.05$). To further examine the localization of EGF receptor in cells expressing N-mVps24, we treated control and N-mVps24p expressing cells with EGF (100 ng/ml, 30 min), acid washed them to remove excess surface ligand, and fixed the cells immediately or after a 120-min incubation. All cells fixed immediately after EGF treatment showed limited surface labeling, which was likely the result of receptor-mediated internalization and a prior acid wash. However, after a 3-h chase, control and nontransfected cells lacked this surface labeling and had limited intracellular labeling consistent with normal degradation. In contrast, cells transfected with N-mVps24p showed intracellular and punctate surface labeling with the EGFR antisera. We next examined the effect of N-mVps24p on EGFR degradation and found that its expression decreased EGF receptor degradation (Fig. 5C). A time course (0–60 min) revealed that the rate of EGFR degradation was decreased (data not shown). These data suggested that expression of N-mVps24p increased the rate of EGF receptor recycling and decreased EGF receptor degradation, and that the recycled receptors can be visualized on the cell surface.

Discussion

The facile genetic approaches in *S. cerevisiae* have identified over 50 genes whose protein products play key roles in the vacuolar/lysosomal and endocytic traffic systems [33,34]. Much molecular detail about protein and lipid transport has emerged from studies of these Vps proteins in yeast. In particular, a number of Vps proteins (the Class E proteins) function in the sorting of integral membrane proteins at multivesicular bodies (MVBs) (e.g., Refs. [32,35]). Identification and characterization of mammalian homologs for all of these yeast proteins can produce new insights into their roles in the endosomal system. For example, mammalian cells contain organelle morphology that provides well-characterized landmarks for localization. Moreover, mammalian cells contain a larger variety of ligands and receptors with which to use as markers for following endocytic pathways in more detail.

Alterations in the steady state distribution of mVps24p and the M6PR (from the trans-Golgi-network to endosomes) were induced by adventitious expression of Hrs or hVps4EQ. Colocalization of mVps24p with Hrs-induced structures indicates that the cellular cycle of mVps24p includes early endosomes. Colocalization of mVps24p with hVps4p-induced structures is consistent with mVps24p, like scVps24p, associating with endosomal membranes in an mVps4/Vps4p-dependent manner [12,18]. These data are also consistent with mVps24p being recruited to the endosomal membrane as part of a larger protein complex. Interestingly, in Mucolipidosis Type II (MLII) cells, hrs did not induce a redistribution of mVps24p or the M6PR, instead, they colocalized on the reticulated TGN, characteristic of MLII cells (Fig. 4). Thus, in the absence of transport from the TGN to endosomes as occurs in MLII, a block in trafficking through early endosomes does not cause a redistribution of mVps24p. These data suggest that mVps24p recruitment is dependent on an intact M6PR-dependent pathway. Furthermore, an additional role for mVps24p, other than that in MVB sorting, can be envisioned that is related to targeting of lysosomal enzymes that have a mannose-6-phosphate signal.

Endocytic pathways potentially influenced by mVps24p given its steady state distribution and the known interactions of the yeast Vps24p include those originating at the endosome (e.g., endosome–lysosome, endosome–plasma membrane) and the TGN (e.g., TGN–endosome). Our data suggest that the endosome–lysosome step(s) are most important and that the increased receptor recycling we observe is the result of the inhibition of this step(s). This increased recycling might be expected for the EGFR when its degradation is blocked and probably reflects a “constipation” effect.

Characterization of mVps24p, the mammalian homolog of the yeast Vps24p, suggests that it plays a role in traffic from the early endosome. For example, the lipid-binding N-terminal of mVps24p domain produces enlarged early endosomes, which confirms the results obtained by Whitley et al. [18]. However, more direct evidence in this study for mVps24p playing a role in traffic at the early endosome is that expression of its lipid binding domain, N-mVps24p, results in an increase in the rate of recycling of the EGF receptor and a decrease in the rate of its degradation. Given the hypothesized function of yeast class E Vps proteins [12,15,32] and other human class E homologs [13,15–17,36,37], it is likely that mVps24p participates in sorting of the EGF receptor at the level of the early endosome and that when its function is compromised the receptor is not properly sorted for degradation and is instead recycled at a greater rate.

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