Molecular Characterization of the Protein Encoded by the Hermansky-Pudlak Syndrome Type 1 Gene*

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Hermansky-Pudlak syndrome (HPS) comprises a group of genetic disorders characterized by defective lysosome-related organelles. The most common form of HPS (HPS type 1) is caused by mutations in a gene encoding a protein with no homology to any other known protein. Here we report the identification and biochemical characterization of this gene product, termed HPS1p. Endogenous HPS1p was detected in a wide variety of human cell lines and exhibited an electrophoretic mobility corresponding to a protein of ~80 kDa. In contrast to previous theoretical analysis predicting that HPS1p is an integral membrane protein, we found that this protein was predominantly cytosolic, with a small amount being peripherally associated with membranes. The sedimentation coefficient of the soluble form of HPS1p was ~6 S as inferred from ultracentrifugation on sucrose gradients. HPS1p-deficient cells derived from patients with HPS type 1 displayed normal distribution and trafficking of the lysosomal membrane proteins, CD63 and Lamp-1. This was in contrast to cells from HPS type 2 patients, having mutations in the β3A subunit of the AP-3 adaptor complex, which exhibited increased routing of these lysosomal proteins through the plasma membrane. Similar analyses performed on fibroblasts from 10 different mouse models of HPS revealed that only the AP-3 mutants pearl and mocha display increased trafficking of Lamp-1 through the plasma membrane. Taken together, these observations suggest that the product of the HPS1 gene is a cytosolic protein capable of associating with membranes and involved in the biogenesis and/or function of lysosome-related organelles by a mechanism distinct from that dependent on the AP-3 complex.

Hermansky-Pudlak syndrome (HPS)† (Online Mendelian Inheritance in Man number 203300) comprises a group of autosomal recessive disorders characterized by oculocutaneous albinism, prolonged bleeding, and progressive pulmonary fibrosis and granulomatous colitis (Ref. 1, reviewed in Refs. 2–4). These symptoms arise from structural and/or functional abnormalities in a group of related organelles, namely melanosomes and platelet dense granules, and lysosomes (2–4). HPS is rare in the general population, but occurs with high frequency in certain isolated groups. For instance, in the northwestern region of Puerto Rico, HPS occurs with a frequency of 1 in 1,800 individuals (5).

The molecular basis for HPS has only recently begun to be unraveled. It is now clear that HPS can arise from mutations in different genetic loci (6–8). The Puerto Rican variant of HPS, termed HPS type 1 (HPS-1), is due to mutations in a gene (HPS1) that encodes a putative protein of 700 amino acid residues and a molecular mass of 79.3 kDa (9). An alternative splicing product of this gene, encoding a putative protein of 324 amino acids and a molecular mass of 36.5 kDa, has also been described (10). The orthologous mouse gene was found to be defective in the pole ear mutant strain (11, 12), which has long been regarded as a murine model of human HPS (reviewed in Ref. 13). Another form of HPS, termed HPS type 2 (HPS-2), has been recently documented in a family of Dutch ancestry (8). These patients were found to bear mutations in the ADTB3A gene encoding the β3A subunit of the heterotetrameric protein complex, AP-3 (8). The mouse orthologue of this gene was found to be defective in the mouse strain pearl, which is also considered an animal model for HPS (14). The AP-3 complex is a member of a family of cytosolic AP complexes involved in coated vesicle formation and signal-mediated sorting of integral membrane proteins within the endocytic and late secretory pathways (15–17). Fibroblasts from HPS-2 patients exhibited a drastic reduction in AP-3 cellular content due to enhanced degradation of its β3A subunit, as well as increased trafficking of lysosomal membrane proteins through the plasma membrane en route to late endosomal/lysosomal compartments (8). It is therefore likely that HPS-2 arises from impairment of protein transport to lysosomes as well as to lysosome-related organelles such as melanosomes and platelet dense granules. Finally, some HPS patients do not bear any detectable mutation in HPS1, ADTB3A, or any other AP-3 subunit gene (8). Obvious candidates for the gene(s) mutated in these patients are those affected in a number of additional mouse models of HPS (13); these genes, however, still remain to be identified.

While the biochemical properties of the AP-3 complex have been extensively studied (18–23), the product of the HPS1 gene has not yet been identified, much less molecularly characterized. The predicted protein (HPS1p) from both humans and mice has no sequence homology to any other known protein (9, 11, 12). Theoretical analysis of the 700-amino acid form of human HPS1p predicted the existence of two membrane-spanning domains comprising residues 79–95 and 369–396 (9). Such a polypeptide would be expected to be a bitopic integral membrane protein, with both the amino- and carboxyl-terminal

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§ The abbreviations used are: HPS, Hermansky-Pudlak syndrome; AP, adaptor protein; HPS-1, HPS type 1, HPS-2, HPS type 2; bp, base pair(s).

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segments (residues 1–78 and 397–700, respectively) facing the cytoplasm and the intervening loop (residues 96–368) facing the luminal/extracellular side of the membrane. Theoretical analyses of the orthologous mouse protein predicted either one (12) or two (11) membrane-spanning domains. The sequences of both human and mouse HPS1p contain several potential dileucine-based motifs (9, 12) similar to signals that mediate transport to late endosomal/lysosomal compartments (24, 25). The presence of such motifs has led to speculation that HPS1p might be targeted to late endosomal/lysosomal compartments, including melanosomes and platelet dense granules. The role that HPS1p would play in these compartments remains unknown.

In this work, we sought to identify and characterize biochemically the endogenous HPS1p from human cells in culture. We report that, contrary to expectations, HPS1p is not an integral membrane protein but exists as both cytosolic and peripheral membrane species. We also show that cells deficient in HPS1p do not display the abnormal trafficking of lysosomal membrane proteins described previously for HPS-2 cells (8) and reported herein for fibroblasts derived from the murine AP-3 mutants (and HPS models), pearl and mocha. These observations, together with the apparent lack of physical interaction between HPS1p and AP-3, suggest that HPS1p controls the biogenesis or function of lysosome-related organelles by a mechanism different from that involving the AP-3 complex.

**Experimental Procedures**

**Cells and Culture Conditions**—Primary cultures of skin fibroblasts (GM00037F and GM000316A) and B-lymphoblastoid cell lines (GM00130C and GM00131B) derived from normal individuals, as well as fibroblasts (GM14609) and B-lymphoblastoid cells (GM14606) from an HPS-1 patient homozygous for a 16-bp duplication in exon 15 of the HPS1 gene (9) were obtained from Coriell Cell Repositories (Camden, NJ). Fibroblasts from a second HPS-1 patient (Patient 8) with identical 16-bp duplication in HPS1, and from an HPS-2 patient (patient 40) with compound heterozygous mutations in the AP-3 complex β3A subunit, have been described previously (8). B-lymphoblastoid cell lines established from patient 40, from a sibling of patient 40 also suffering from HPS-2 (patient 39), and from three unaffected relatives of patients 40 and 42 who are heterozygous carriers of the AP-3 β3A ∆390–410 deletion, have also been described (8). Culture conditions for fibroblasts and B-lymphoblasts are indicated elsewhere (8).

Fibroblasts from various mouse strains were isolated by incubating, in 15-mm diameter plastic dishes, minced endodermis with complete Dulbecco’s modified Eagle’s medium (Dulbecco’s modified Eagle’s medium supplemented with 20% (v/v) fetal bovine serum, 2 mM glutamine, 100 μg/ml streptomycin, 100 IU/ml penicillin) for 3 days in a standard CO2 incubator. Adherent cells were then expanded by repeated passages in the same medium.

The sources and culture conditions for the human cell lines M1, HeLa, H4 and Jurkat have been indicated in a previous report (19). Megakaryocyte-derived MEG-01 cells and intestinal epithelial Caco-2 cells were from the American Tissue Culture Collection (Manassas, VA). MEG-01 cells were grown in suspension in RPMI 1640 medium supplemented with 15% (v/v) fetal bovine serum, 2 mM glutamine, 100 μg/ml streptomycin, and 100 IU/ml penicillin. Caco-2 cells were propagated in complete Dulbecco’s modified Eagle’s medium supplemented with non-essential amino acids and 1 mM sodium pyruvate. Melanoma-derived MNT-1 cells were a gift from Dr. Vincent Hearing (NIH, Bethesda, MD) and cultured in a medium similar to that of Caco-2 cells with the addition of 10% (v/v) AIM-V medium (Life Technologies, Inc., Gaithersburg, MD) and fungizone.

**Antibodies**—The peptide sequence DDIQPSPRRARSSQN, corresponding to residues 253–267 of human HPS1p (9) was used to generate a polyclonal rabbit antibody (Zymed Laboratories Inc., San Francisco, CA). The antibody was affinity purified (26) using the peptide as a ligand and 0.1 M glycine, pH 2.5, as elution buffer. Monoclonal rat antibody to mouse Lamp-1 (1D4B) was from the Developmental Studies Hybridoma Bank at the University of Iowa (Iowa City, IO). Monoclonal antibody to Hisα tag was purchased from CLONTECH (Palo Alto, CA). The sources for all the other antibodies used in this study have been indicated elsewhere (8).

**Biochemical Procedures**—Metabolic labeling of cultured cells with [35S]methionine, preparation of Triton X-100 extracts, preparation and ultracentrifugation of detergent-free extracts, salt extraction of post-nuclear membranes, sucrose gradient fractionation, and immunoprecipitation-recapture were performed as described (18). Further details of protein microscopy and immunoprecipitation-recapture method are provided elsewhere (27). The following proteins were used as standards for sedimentation velocity experiments (s20,w, values given in parentheses): chicken ovalbumin (36.8 S), bovine serum albumin (4.6 S), bovine catalase (11.3 S), and horse spleen ferritin (16.5 S).

**Nuclear Acid Manipulations**—Total RNA was isolated from cells in culture using the TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD). Reverse transcriptase-polymerase chain reaction was carried out using the Superscript kit from Life Technologies, Inc. with 5’ and 3’ primers comprising codons 495–502 and complementary to codons 694–701 of the predicted open reading frame of human HPS1 (9), respectively.

A construct encoding a Hisα-HPS1 p fusion protein was generated by cloning in-frame the open reading frame of human HPS1 cDNA into the KpnI-XbaI sites of the pcDNA3.1HisA vector (Invitrogen, Carlsbad, CA). The sequence of the construct was verified by DNA sequencing.

**DNA Transfection, Immunofluorescence, Antibody Internalization, and Flow Cytofluorometry**—Human HeLa or M1 cells grown on glass coverslips were transfected with the Hisα-HPS1p construct by using the FuGENE-6 reagent (Roche Molecular Biochemicals, Indianapolis, IN). Two days after transfection, cells were either fixed and processed for immunofluorescence (18) or used directly for antibody internalization experiments, as described (8). Quantitation of antibody internalization in human or mouse fibroblasts and flow cytofluorometry of B-lymphoblasts were also performed as described previously (8).

**Results**

**Identification of HPS1p**—To analyze the properties of HPS1p, we generated an affinity-purified, rabbit polyclonal antibody to residues 253–267 of its deduced amino acid sequence (9). This antibody failed to detect endogenous HPS1p from HeLa cells or M1 fibroblasts by either immunoblotting or immunofluorescence microscopy, but could detect using the same methods a Hisα-HPS1p fusion protein expressed by transfection in these cells (data not shown; see, for example, Fig. 4A).

In order to detect the endogenous protein, we resorted to an immunoprecipitation-recapture protocol, which had been used successfully to study low-abundance cellular proteins (e.g. see Ref. 28). HeLa cells metabolically labeled with [35S]methionine were extracted with a Triton X-100-containing buffer, and the resulting extract was subjected to nondenaturing immunoprecipitation with the anti-HPS1 antibody. Proteins in the immunoprecipitate were then denatured by heating in the presence of SDS and dithiothreitol, diluted 20-fold in Triton X-100 buffer containing iodoacetamide (to quench excess dithiothreitol), and subjected to a second immunoprecipitation with the same anti-HPS1 antibody. This procedure resulted in the isolation of a major radiolabeled protein of apparent molecular mass ~80 kDa (Fig. 1A), a value which was in close agreement with the calculated mass of the largest predicted HPS1p species (79.3 kDa, Ref. 9). A minor species of slightly higher electrophoretic mobility was also observed (Fig. 1A); the nature of this species remains to be determined. Specificity controls confirmed that the species recognized by the anti-HPS1 antibody represented endogenous HPS1p. First, no bands were detected using two irrelevant control antibodies in the second immunoprecipitation (Fig. 1A).

In addition, virtually no protein bands were detected when the immunoprecipitation-recapture procedure was performed on fibroblasts from two unrelated HPS-1 patients. However, antibodies to residues of a 16-bp duplication in HPS1 (Fig. 1B); this mutation had been shown to affect the stability of the HPS1 mRNA (9, 29) and predicted to result in very low cellular levels of HPS1p. Fibroblasts from an AP-3 β3A-deficient HPS-2 patient were found to express normal levels of HPS1p (Fig. 1B) and immunoprecipitation-recapture experiments failed to reveal interaction between HPS1p and the AP-3 complex (Fig. 1B).
Characterization of HPS1p

HPS1p is Mainly a Cytosolic Protein—To examine the subcellular localization of HPS1p, [35S]methionine-labeled HeLa or M1 cells were mechanically disrupted in the absence of detergents and subjected to a simple fractionation procedure in which a post-nuclear supernatant was centrifuged for 90 min at 120,000 × g, and the resulting supernatant and pellet fractions were analyzed for the presence of HPS1p by immunoprecipitation-recapture. Following this procedure, approximately 70–80% of endogenous HPS1p was recovered in the supernatant, while the remainder was associated with the post-nuclear pellet (Fig. 3A). Similar results were obtained when using repetitive ultracentrifugation steps to sediment any contaminating membranes that could remain in the 120,000 × g supernatant (data not shown). As expected, the lysosomal integral membrane protein, Lamp-1, was found exclusively in the post-nuclear pellet fraction (Fig. 3A). The small amounts of HPS1p associated with membranes could be solubilized by extraction with 2 M NaCl (Fig. 3B), indicating that this fraction of HPS1p behaved as a peripheral membrane protein. To estimate the size of the soluble form of HPS1p, a cytosolic extract of [35S]methionine-labeled HeLa cells was fractionated by ultracentrifugation on a sucrose density gradient, and the resulting fractions were subjected to immunoprecipitation-recapture with the anti-HPS1 antibody. As shown in Fig. 3C, HPS1p was recovered in fractions corresponding to a sedimentation coefficient of ~6 S. In agreement with these biochemical properties of endogenous HPS1p, immunofluorescence staining of HeLa or M1 cells expressing a His6-HPS1p fusion protein resulted in a largely diffuse, cytoplasmic pattern (Fig. 4, A and C, and data not shown). Taken together, these results suggested that HPS1p exists both as a soluble protein and as a peripheral membrane protein.

Analysis of a Possible Functional Interaction Between HPS1p and AP-3—Because mutations in the genes encoding HPS1p or the β3A subunit of AP-3 result in a similar syndrome (i.e. HPS) it was of interest to determine whether HPS1p interacted functionally with AP-3. We had previously shown that inactivation of the HPS1 gene did not affect the cellular content of the AP-3 complex or its intracellular distribution (8). As shown in Fig. 4, the characteristic localization of AP-3 to punctate foci within the cytoplasm was also unaffected by overexpression of a His6-HPS1p fusion protein in M1 fibroblasts. Together, these results deemed unlikely the possibility of HPS1p being a regulator of the association of AP-3 to membranes, a function that had been previously ascribed to the small GTPase, ARF1 (23, 30).

In a previous study (8), AP-3-deficient cells from HPS-2 patients were shown to exhibit increased trafficking of lysosomal membrane proteins (e.g. CD63 and Lamp-1) through the

FIG. 1. Detection of endogenous HPS1p by immunoprecipitation-recapture. A, HeLa cells were metabolically labeled with [35S]methionine for 22 h and extracted with lysis buffer containing 1% (w/v) Triton X-100 (18). The extract was then subjected to a first immunoprecipitation (1st IP) with anti-HPS1 antibody. The immunoprecipitate was subsequently heated at 95 °C for 5 min in the presence of SDS and dithiothreitol, diluted 20-fold with lysis buffer, and subjected to a second immunoprecipitation (2nd IP) with the same anti-HPS1 antibody or with either of two different control antibodies. Proteins were then analyzed by 4–20% gradient SDS-polyacrylamide gel electrophoresis and fluorography. The positions of molecular mass markers are indicated on the left. B, whole cell extracts from primary cultures of fibroblasts derived from a normal individual, two patients homozygous for a 16-bp duplication in the HPS1 (HPS-1) and a patient with compound heterozygous mutations in the β3A subunit of AP-3 (HPS-2), were prepared and analyzed as described in A by using in both immunoprecipitation and recapture steps the antibody to HPS1p (upper panel) or an antibody to a control cellular protein (lower panel). C, immunoprecipitation-recapture experiment, performed as in A, in which antibodies to HPS1p (this study) or the α3 subunit of AP-3 (18) were used alternatively for the first or second immunoprecipitations. Notice that the HPS1p and AP-3 α3 proteins were recovered only when their respective antibodies were used in both immunoprecipitation steps.

FIG. 2. Expression of endogenous HPS1p and HPS1 mRNA in various human cells lines. The cell lines indicated on the figure were analyzed for expression of HPS1p by metabolic labeling followed by immunoprecipitation-recapture (upper panel) or for HPS1 mRNA expression by reverse transcriptase-polymerase chain reaction followed by agarose gel electrophoresis and ethidium bromide staining (lower panel). For immunoprecipitation-recapture, Triton X-100 extracts from the different cell lines were normalized for the total amounts of [35S]methionine incorporated into proteins, as determined by trichloroacetic acid precipitation (42).

Previous Northern blot experiments suggested that the HPS1 mRNA is expressed in most cell types, although it was barely detectable in HeLa cells (9). We analyzed a panel of human cell lines for expression of HPS1 at both the mRNA and protein levels. As shown in Fig. 2, both the protein and the mRNA were detected in a variety of human cell lines, including M1 (fibroblast), MNT-1 (melanoma), H4 (neuroblastoma), MEG-01 (megakaryocyte), Jurkat (T cell), and Caco-2 (intestinal epithelial). Although HPS1p levels were variable among the different cell lines, we did not find a cell line that expressed levels much higher than those observed in HeLa cells (Fig. 2). Of particular interest was the fact that the amount of HPS1p was not significantly higher in a melanoma and a megakaryocyte cell line, both of which are derived from cell types where the HPS1p deficiency gives rise to characteristic manifestations of HPS (i.e. deficiency in pigmented melanosomes and platelet dense granules). These experiments thus suggested that HPS1p is expressed ubiquitously among human cells.

1C), suggesting that HPS1p and AP-3 are not part of a stable complex.
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Fig. 3. HPS1p exists in soluble and membrane-associated forms. A, HeLa and M1 cells were metabolically labeled with [35S]methionine for 22 h, mechanically disrupted by successive passages through a 25-gauge needle, and centrifuged for 5 min at 800 × g to remove intact cells and nuclei. The post-nuclear supernatant was then ultracentrifuged at 4 °C for 90 min at 120,000 × g, to yield supernatant (S120) and pellet (P120) fractions. The pellet was solubilized with buffer containing 1% (w/v) Triton X-100 and the supernatant was brought up to the same concentration of Triton X-100 by addition of an equal volume of 2× concentrated buffer. Subsequently, HPS1p was isolated by immunoprecipitation-recapture using the anti-HPS1 antibody as described in the legend to Fig. 1, and the lysosomal integral membrane protein, Lamp-1, was isolated by a single immunoprecipitation-recapture step with the HA43 monoclonal antibody. B, immunoprecipitation-recapture of supernatants (S) and pellets (P) generated by overnight extraction of a P120 membrane pellet obtained as in A with a low salt buffer (18) or with the same buffer containing 2 mM NaCl, followed by ultracentrifugation at 4 °C for 90 min at 120,000 × g. C, sedimentation velocity analysis of an S120 soluble fraction obtained as in A with a low salt buffer followed by ultracentrifugation on a 5–20% (w/v) sucrose gradient (18) followed by immunoprecipitation-recapture with an antibody to HPS1p. The positions of standard proteins on the sucrose gradient are indicated on the top.

Fig. 4. Distribution of the AP-3 complex in cells overexpressing HPS1p. M1 fibroblasts were transfected with a DNA construct encoding His6-HPS1p and co-stained with a rabbit anti-HPS1 antibody (A) and a mouse polyclonal antibody (28) to the AP-3 complex (B) followed by Alexa448-conjugated anti-rabbit IgG and Cy3-conjugated anti-mouse IgG. Alternatively, cells were co-stained with a mouse monoclonal to the His6 tag (C) and the b3C1 rabbit antibody (19) to AP-3 β3A (D) followed by Alexa448-conjugated anti-mouse IgG and Cy3-conjugated anti-rabbit IgG. Notice the cytoplasmic diffuse pattern of the expressed His6-HPS1p fusion protein (A and C) and the characteristic punctuate pattern of endogenous AP-3 (B and D).

Transferring Lysosomal Membrane Proteins in Fibroblasts from Marine Models of HPS—The experiments described in the previous section suggested that the different forms of HPS could be classified according to their cellular trafficking phenotype (i.e. whether the routing of lysosomal membrane proteins through the plasma membrane was abnormally increased). This led us to examine the phenotype of cells derived from a panel of mutant mice that display the characteristic symptoms of HPS (i.e. defective melanosomes and platelet dense granules; Ref. 13). We obtained fibroblasts from the strains mocha and pearl, which bear mutations in the δ and β3A subunits of the AP-3 complex, respectively (14, 31), as well as from pallid, cocoa, muted, sandy, reduced pigmentation, ruby eye, ruby eye-2, and light ear, for which the mutated genes remain to be identified. In agreement with previous reports (31, 32), fibroblasts from mocha and pearl displayed drastically reduced levels of AP-3 subunits (data not shown). On the other hand, none of the other mutant strains exhibited abnormal AP-3 protein content or intracellular distribution (data not shown). As shown in Fig. 7, enhanced internalization of an anti-Lamp-1 antibody was observed in cells from mocha and pearl mice. This was consistent with the phenotype observed for AP-3-deficient fibroblasts from HPS-2 patients (Ref. 8 and Fig. 6) or for AP-3-deficient cells obtained by using an antisense approach (33). In contrast, internalization of anti-Lamp-1 antibodies in cells from the other HPS-like mutant mice was similar, or even reduced, as compared with that in cells from wild-type controls (Fig. 7).
patients and the normal levels in the HPS-1 patient cells. Notice the increased surface levels of CD63 in cells from the two HPS-2 patients who bear a single mutated allele (AP-3 subunit (AP-3βA)), and three unaffected relatives of the HPS-2 patients who bear a single mutated allele (AP-3βA heterozygous) (8). Notice the increased surface levels of CD63 in cells from the two HPS-2 patients and the normal levels in the HPS-1 patient cells.

**DISCUSSION**

We have identified the product of the *HPS1* gene as an ~80 kDa protein that is expressed in a wide variety of human cell types. Identification of the endogenous protein was made possible by immunoprecipitation-recapture, using an affinity-purified antibody directed to a sequence from the predicted product of the *HPS1* gene. The specificity of detection of HPS1p by this method was demonstrated by using irrelevant control antibodies (Fig. 1A) and, more importantly, by applying the immunoprecipitation-recapture procedure to whole cell extracts from HPS-1 patient fibroblasts (Fig. 1B), which had been predicted to express little or no HPS1p due to enhanced degradation of the HPS1 mRNA (9, 29).

The electrophoretic mobility of endogenous HPS1p was consistent with the molecular mass predicted for the product of the major HPS1 transcript (9). An alternatively spliced form of the HPS1 mRNA, encoding a putative 36.5-kDa protein, had also been described (10). Although the sequence of this putative 36.5-kDa form of HPS1p is predicted to include the amino acid segment used to generate our anti-HPS1 antibody, we have been unable to detect by immunoprecipitation-recapture such a form in the human cells lines analyzed (e.g., Fig. 1, A and B).

Subcellular fractionation experiments revealed that the majority of endogenous HPS1p exists as a ~6 S species in the cytosol, with a smaller fraction being peripherally associated with membranes. Although we cannot rule out that the fractionation procedure could have caused dissociation of the protein from membranes, immunofluorescence microscopy of HeLa and M1 cells transfected with an epitope-tagged HPS1p construct showed a diffuse cytoplasmic staining pattern characteristic of a cytosolic protein. The subcellular distribution of HPS1p is, therefore, in accordance with the absence of an amino-terminal signal peptide for translocation across the membrane of the endoplasmic reticulum, while it is at variance with theoretical analyses using either the Engelman-Steitz-Goldman hydrophilicity scale (34) or the TMpred program (35), which had predicted a protein with either one or two membrane-spanning helices, respectively (9, 11, 12). Interestingly, analyses using the TMHMM program based on hidden Markov models (36), or using the hydrophathy scale of Kyte and Doolittle (37), predict that HPS1p contains no transmembrane domains (data not shown), in agreement with our experimental data. The above discrepancies between the structural predictions made by different methods underscore the need to validate theoretical predictions with biochemical analyses of the protein.

Although the precise biological function of HPS1p is unknown, its association with HPS in humans and mice argues for a role in the biogenesis and/or function of lysosomes, melanosomes, and platelet dense granules. Recent work has established that the heterotetrameric AP-3 adaptor complex is also associated with this disorder (8, 14, 31). AP-3 is a known component of the cellular machinery that mediates signal-dependent sorting of integral membrane proteins within the endocytic and late secretory pathways (15, 16, 17). In addition, recent evidence has suggested a role for AP-3 in the trafficking of a subset of transmembrane proteins that normally localize to lysosomes, melanosomes, and platelet dense granules (e.g., CD63 and Lamp-1) (8, 33). These findings have led to speculation on possible roles for HPS1p in AP-3-dependent trafficking events. In this regard, it is worth mentioning that the well characterized AP-2 adaptor complex, which is structurally re-
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Fig. 7. Trafficking of Lamp-1 through the surface of fibroblasts from mouse models of HPS. Fibroblasts derived from two control mouse strains (wt) and the indicated mouse strain models of HPS were grown on glass coverslips and then allowed to internalize a rat monoclonal antibody ID4B to mouse Lamp-1 for 15 min at 37 °C. Subsequently, cells were washed for 5 min in ice-cold phosphate-buffered saline, fixed in 2% formaldehyde for 10 min, permeabilized, and then stained with Cy3-conjugated anti-rat IgG. Fluorescence quantitation was performed as summarized in the legend to Fig. 6 and described in more detail in a previous study (8). Background-corrected values from two to six independent experiments are expressed in arbitrary units of fluorescence per cell (mean ± S.D.).

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