MOLECULAR GENETICS AND METABOLISM 65, 85–96 (1998)
ARTICLE NO. GM982729

4

MINIREVIEW

Hermansky-Pudlak Syndrome: Models for Intracellular Vesicle Formation

Vorasuk Shotelersuk and William A. Gahl[1]

Section on Human Biochemical Genetics, Heritable Disorders Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

Received May 20, 1998, and in revised form June 1, 1998

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder characterized by pigment dilution, nystagmus, decreased visual acuity, a bleeding diathesis, and lysosomal accumulation of ceroid lipofuscin. Electron microscopic evidence demonstrating lack of platelet-dense bodies provides the *sine qua non* for diagnosing HPS. Ceroid lipofuscinosis is considered to cause several serious complications, including progressive pulmonary fibrosis leading to death in the fourth or fifth decades. Currently, only symptomatic treatment can be offered. Although rare in the general population, HPS occurs in northwest Puerto Rico with a prevalence of 1 in 1800. *HPS1*, the first gene found to be responsible for HPS, was mapped to chromosome 10q23 and subsequently isolated and sequenced. It consists of 20 exons encoding a 700-amino acid, 79.3-kDa peptide with no homology to any known protein. All 10 *HPS1* mutations reported to date, including the 16-bp duplication found in all northwest Puerto Rican patients, result in truncated proteins. The two mutations in the mouse *pale ear* gene (*ep*), which is the murine homology of *HPS1*, cause similarly truncated proteins. The pathologic nature of these truncation mutations may result from unstable mRNA. However, in combination with the absence of any disease-causing missense mutations, it may indicate that the C-terminus of the HPS1 peptide is functionally important. The disorder HPS displays locus heterogeneity, consistent with the existence of 14 mouse strains manifesting both hypopigmentation and a platelet storage pool deficiency. Two mouse models, *pearl* and *mocha*, have mutations in the β3A and δ subunits of the adaptor-3 complex, respectively. This suggests that defective vesicular trafficking, specifically cargo packaging, vesicle formation, vesicle docking, or membrane fusion, may comprise the basic defect in HPS. Studies of the proteins involved in intercompartmental transport for melanosomes, platelet-dense bodies, and lysosomes should lead to a better understanding of the mechanisms of organellogenesis and to more effective therapies for HPS. © 1998 Academic Press

Key Words: **albinism; platelet storage pool deficiency; vesicle trafficking; membrane fusion; ceroid lipofuscin.**

In 1959, two Czechoslovakian physicians described two patients who manifested a constellation of findings including oculocutaneous albinism and a platelet-related bleeding diathesis (1). The syndrome eventually assumed the physicians' names and became recognized as a rare genetic entity defined by the combination of pigment dilution and a platelet storage pool defect (2,3). It was also characterized by presumably lysosomal accumulation of a lipid-protein material known as ceroid lipofuscin. In practical terms, Hermansky-Pudlak syndrome (HPS) came to be diagnosed by the absence of platelet-dense bodies on electron microscopy in a hypopigmented patient with a bleeding diathesis; the

[1] To whom correspondence should be addressed at 10 Center Drive, MSC 1830, Building 10, Room 9S-241, NICHD, NIH, Bethesda, MD 20892-1830. Fax: (301) 402-0234. E-mail: bgahl@helix.nih.gov.

1096-7192/98 $25.00

demonstration of ceroid lipofuscin is not required. Although the basic defect in HPS is not known, it is considered to involve a vesicular membrane shared by the melanosome, dense body, and lysosome, all of which are affected in HPS. The discovery of one HPS-causing gene, i.e., *HPS1* (4), and its homologue, *ep,* in the mouse model *pale ear* (5,6), may lead us to the basic defect, but the function of the *HPS1/ep* protein product has not yet been determined. Mutations in genes other than *HPS1* undoubtedly result in the clinical manifestations of HPS (7–9); the various genes responsible for this disorder may well contribute to different steps in a common pathway of intracellular vesicle formation and trafficking. The present review describes HPS from clinical and molecular perspectives and promotes this heterogeneous disorder as a model for investigations into vesicular trafficking and organellogenesis.

PREVALENCE

HPS is an autosomal recessive disorder that exists in a wide variety of ethnic groups (2,3). Over the past two decades, it has been recognized that HPS occurs very commonly in northwest Puerto Rico (10), where it affects approximately 400 individuals and has a prevalence of 1 in 1800, apparently due to a founder effect. A Swiss isolate has also been described (11,12), and consanguineous patients have been reported in Japan (4). Approximately 100 non-Puerto Rican patients are known in the United States, but this probably represents an underestimate due to a low index of suspicion for this disorder, with consequently poor ascertainment.

CLINICAL MANIFESTATIONS

Albinism

In general, the oculocutaneous albinism of HPS is recognized at birth by the presence of hypopigmentation and horizontal nystagmus. The degree of hypopigmentation of the skin, hair, iris, and retinal fundus varies extensively, even in patients homozygous for the same mutation (12). The skin, when exposed to intense sunlight, as in Puerto Rico, acquires a typical appearance characterized by nevi, actinic keratoses, and sun damage (Fig. 1A). Patients are at risk for basal cell carcinoma and other dermatologic disorders, including ephelids and lentigines (Toro J, Turner M, Gahl WA. Dermatologic manifestations of HPS in patients with and without a 16-bp duplication in the *HPS* gene. Submitted for publication, 1998). The hair color can range from dark (7) to completely white (Fig. 1B), but the typical patient has a tan/blond color which is quite distinctive (Fig. 1C). An occasional patient reports darkening of hair color during childhood. Both Puerto Rican and non-Puerto Rican patients exhibit a wide range of skin and eye pigmentation (13).

The ophthalmic findings of HPS are also variable (13–15). Visual acuity in the better eye varies from 20/50 to 20/250, and does not usually change with age. (Legal blindness in the United States is 20/200 or worse.) Corrective lenses are only occasionally beneficial to patients. Nystagmus, which occasionally has a rotatory component in HPS, accompanies the decreased visual acuity. Foveal hypoplasia reflects decreased retinal pigment and may be the cause of photophobia in affected patients. Iris transillumination documents the absence or paucity of iris pigment. As for other patients with albinism, individuals with HPS generally manifest decreased decussation of optic nerve fibers.

Platelet Storage Pool Deficiency

In HPS, a bleeding diathesis results from a platelet storage pool deficiency. Although there are normal numbers of platelets (2), their dense bodies, which contain ADP, ATP, calcium, and serotonin, are virtually absent in HPS, and this provides the *sine qua non* for diagnosis (16). Dense bodies are intracellular vesicles which disgorge their contents upon platelet stimulation, causing platelet aggregation and clot formation. This secondary aggregation response is impaired in HPS patients (2,3), who usually have a prolonged bleeding time despite normal coagulation factors and a normal or increased platelet count. In general, HPS patients experience mild bleeding events, including bruising, epistaxis, gingival bleeding, prolonged bleeding during menstruation or after tooth extraction or circumcision, postpartum hemorrhage, and bleeding colitis. In one study (13), 20 of 49 HPS patients had major bleeding events, some of which were life-threatening. Childbirth can be dangerous, and a variety of traumatic and surgical events can precipitate exsanguination in severely affected patients. Just as the degree of hypopigmentation is not uniform in all patients, the severity of the bleeding diathesis in HPS varies substantially.

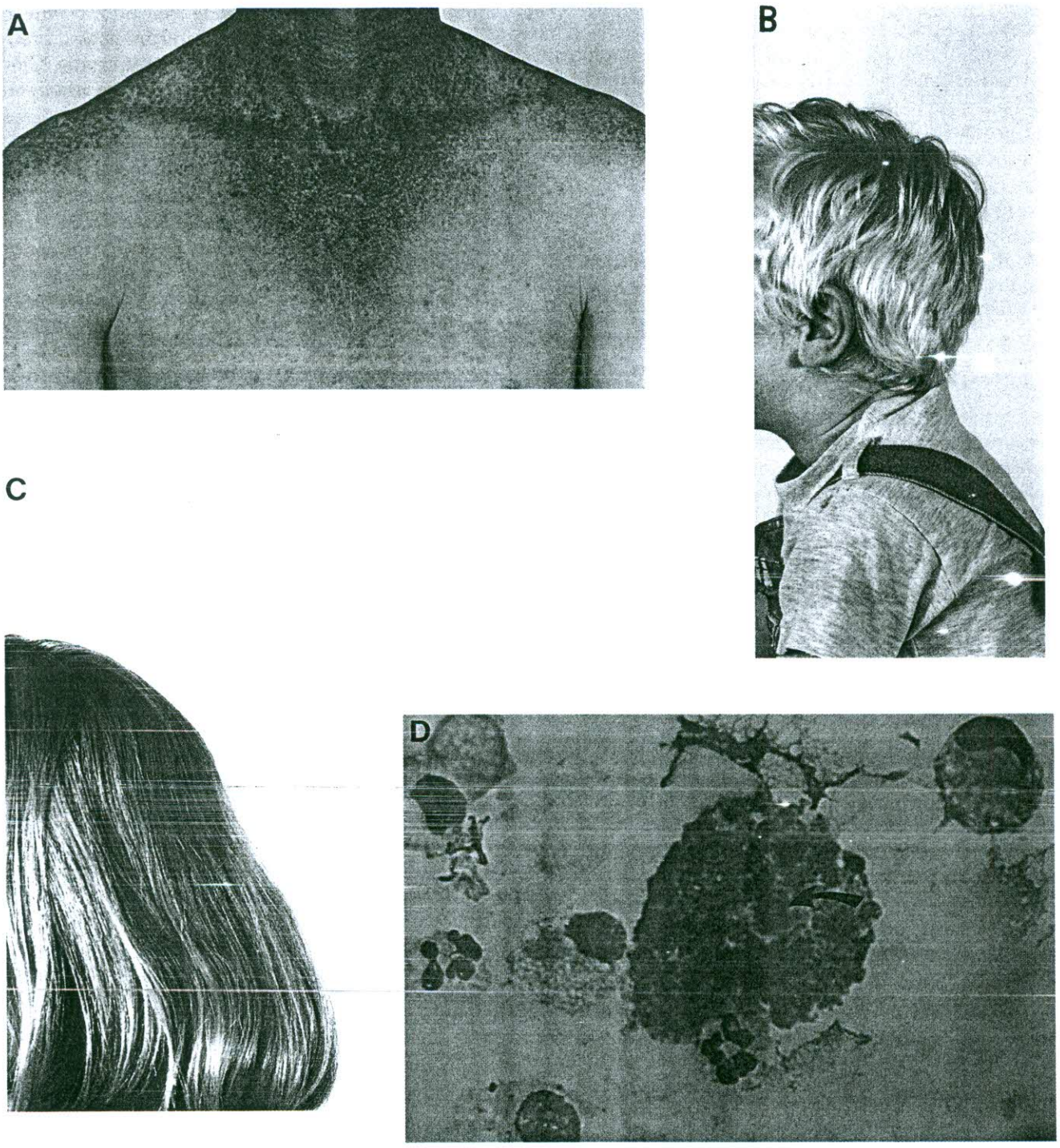

FIG. 1. (A) Solar damage in a 34-year old Puerto Rican patient homozygous for the 16-bp duplication in *HPS1*. (B) White hair of a 3-year old boy homozygous for the 16-bp duplication. (C) Tan/blond hair in a 6-year old girl with HPS, the sister of the boy in Fig. 2A. (D) Alveolar macrophage from the pulmonary lavage of a Puerto Rican adult with HPS. The arrow points to ceroid lipofuscin. (Courtesy of Dr. Mark Brantly, National Heart, Lung, and Blood Institute)

Ceroid Lipofuscinosis

Lysosomal storage of ceroid lipofuscin completes the triad of findings in HPS. Ceroid lipofuscin (Fig. 1D) is a poorly-defined, granular, electron-dense, lipid-protein complex (2) which autofluoresces with a characteristic bright yellow color. In HPS patients with increased ceroid lipofuscin, dolichols are present in the urinary sediment (17), suggesting that these isoprenoids compose at least one component of the storage material. The accumulation of ceroid lipofuscin occurs only sporadically among individuals with HPS and progresses very gradually with age even in classic, Puerto Rican patients (2,17). The tissues of greatest accumulation are the kidney, bone marrow, spleen, liver, and large intestine, but sloughed urinary epithelial cells also contain ceroid lipofuscin. Moderate amounts have been found in lung, heart, and lymph nodes, and lesser amounts scattered in other parenchymal tissues (2). There exist animal models for studying this storage material (18).

Several serious complications of HPS are considered to result from ceroid lipofuscin accumulation. The most devastating is pulmonary fibrosis (19,20), which has occurred in HPS patients of several different ethnic groups, including one of the original Czechoslovakian patients (1), a Japanese male (21), a Belgian (22), and an English family (23). However, members of the northwest Puerto Rican isolate of HPS have a particular susceptibility to this complication (13). The pulmonary fibrosis usually proves fatal in the fourth or fifth decade, but affects patients in an extremely variable fashion. Typically, a slowly progressive, restrictive lung disease worsens rapidly in the last few years of life. The time from first significant pulmonary symptoms to death may be as short as 2–3 years, although a more chronic course is the rule.

In addition to pulmonary fibrosis, bleeding granulomatous colitis resembling Crohn's disease (2,24,25) occurs in approximately 15% of patients (13), and gingivitis, cardiomyopathy, and renal failure have also been reported (2). The immunologic function of lymphocytes and neutrophils appears normal (26).

THERAPY

Although the visual deficits of HPS are irreversible, a few patients benefit from corrective lenses. Standard assistance for the visually impaired should be provided. Bioptic lenses allow some patients to read more easily and, in rare cases, even to drive. There is no therapy for the nystagmus. Clothing and sunscreens offer protection from ultraviolet light, and surveillance against skin cancer is essential.

Regarding the platelet storage pool deficiency, acetylsalicylic acid and aspirin-containing drugs must be assiduously avoided (2). Some HPS patients respond to 1-desamino-8-D-arginine vasopressin (DDAVP) with correction of their bleeding times (27), but in general the bleeding time does not reflect the clinical bleeding tendency. DDAVP (0.2 μg/kg IV in 50 ml of normal saline over 30 min) can be administered prophylactically for dental procedures or elective, minor surgeries. However, platelet and blood transfusions may be required for acute, substantial bleeds. Women often benefit from use of birth control pills to provide regular, controlled menstrual cycles. Clearly, early recognition of the storage pool deficiency in HPS patients is critical in preventing unexpected bleeding episodes. A medical alert bracelet allows for anticipatory treatment in the case of emergencies.

Therapy of the other complications of HPS is largely symptomatic. The granulomatous colitis resembles Crohn's disease (24,25). Although steroids may offer healing and relief, several patients have required colostomies (13). No effective therapy exists for the pulmonary fibrosis of HPS, although high-dose steroids are generally administered in the terminal stages of the process and oxygen provides some relief from the pain of dyspnea. Cigarette smoke and other pulmonary irritants must be religiously avoided, and pneumococcal and yearly influenza vaccinations constitute appropriate prophylactic measures. Moderate exercise should be encouraged. Older patients with HPS should be examined regularly for renal glomerular disease, an uncommon complication.

MOLECULAR ASPECTS

The HPS1 Gene

In 1995, the approach of linkage disequilibrium was utilized to localize an HPS-causing gene to chromosome 10q23 by two independent groups using families from northwest Puerto Rico and an isolated Swiss village (28,29). By positional cloning, the gene, originally named *HPS* but called *HPS1* in this review, was subsequently identified; its cDNA se-

quence revealed an open reading frame of 2100 bp (4). Recently, the genomic structure of *HPS1* was delineated (30). *HPS1* consists of 20 exons spanning approximately 30.5 kb, with two putative transmembrane regions. The mouse homologue of *HPS1* has an intervening sequence 16 which is a member of the very rare U12-type "AT-AC" class of introns (31), making *HPS1* the first human disease-causing gene to claim this distinction (6).

A considerable amount of information is available concerning the *HPS1* gene. First, 18 nonpathologic DNA sequence polymorphisms have been reported (8,30), four of which result in amino acid substitutions (G283W, P491R, R603Q, and V630I). Second, four alternative splices have been described (4,30). The most common, found in nearly half of the HPS cDNA samples studied, is a splice removing the 99-bp exon 9 and resulting in a protein lacking amino acids 257–289 but retaining an intact carboxy terminus (30). A different, rare splice in the coding region adds 43 nucleotides of the donor site of IVS 6, resulting in a frameshift. Two additional alternative splices occur in untranslated regions of the *HPS1* transcript, with one adding 9 nucleotides in the 5′ untranslated region and the other removing 194 nucleotides from the 3′ untranslated region. Finally, we know that there are several normal *HPS1* gene transcripts. Minor 3.9- and 4.4-kb mRNAs are apparent on Northern blot analysis, but the standard transcript is 3.0 kb and is expressed in most tissues (4). This suggests that the *HPS1* gene product may be a component of the lysosomal membrane, which exists in most cells, since melanosomes and dense bodies are present only in melanocytes and platelets, respectively. An additional 1.5-kb transcript with the same 5′ sequence as the published cDNA but with a different 3′ sequence has been found in bone marrow and melanoma cells (32).

The *HPS1* protein consists of 700 amino acids whose predicted composite molecular weight is 79.3 kDa (4). The sequence has no homology to any known protein. The polypeptide contains two presumed transmembrane domains at residues 79–95 and 369–396, two potential N-linked glycosylation sites at residues 528 and 560, and a putative melanosomal localization signal, PLL, at the precise carboxy terminus of the protein. However, the absence of this motif in *ep,* the murine homologue of *HPS1* (5,6), raises doubts about its significance. At residues 433–440, the *HPS1* protein contains the sequence DKF($^{L}/_{V}$)KNRG, which is homologous to the Chediak Higashi disease (CHD) protein (4,33). CHD is a multiorganellar disorder similar to HPS with defective trafficking of secretory lysosomes and a susceptibility to infections (34). The homology suggests an intracellular location or function shared by the *HPS1* and CHD proteins (35).

Mutations in the HPS1 Gene

To date, 10 allelic mutations resulting in 11 different *HPS1* genotypes have been reported (Table 1), with two populations displaying a founder effect. A 16-bp duplication within exon 15 has been found exclusively in patients from northwest Puerto Rico (4,7–9,13), and the T322insC mutation appears in an isolated village in the Swiss Alps (4). Three mutations have been found in different populations. T322insC, apparently the most common *HPS1* gene mutation among non-Puerto Rican patients, has been reported in 5 families with Swiss, Italian/German/Ukrainian, Irish/German, French, and Scottish heritage (4,8,9). Haplotype analysis, performed with the aid of intragenic polymorphisms, indicates that the T322insC mutation arose at least twice in northern Europe (9). A T322delC mutation has been found in two families of German and Japanese ancestry (8,9), and S396delC has appeared in three families of Ukrainian, Dutch/German, and Irish/English/French/Norwegian background (8,9). The region of codons 321–324, and the T322 codon in particular, appears to be a mutation hot spot (9), and codon 396 may be another area subject to recurrent mutation (8,9).

Virtually all *HPS1* gene mutations discovered in humans, as well as the two known, frameshift mutations in the *pale ear* mouse (6), result in a truncated protein. This suggests that the carboxy terminal portion of the *HPS1* polypeptide is critical for function, an hypothesis supported by the fact that no missense mutation has yet been reported. Theoretically, a missense mutation in *HPS1* could be lethal *in utero,* or could cause a phenotype different from HPS as we know it. However, the most likely explanation for the large number of polymorphisms found in *HPS1* is that missense mutations result in a clinically normal phenotype; single base changes may not cause pathologic alterations in the protein's function.

It has been proposed (4) that mutant *HPS1* peptides containing the two putative transmembrane domains (residues 79–95 and 369–396) are particularly damaging because they are incorporated into target membranes. According to this hypothesis,

TABLE 1
Mutations in the *HPS1* Gene

Case	Ethnicity[a]	Mutations	Exons	Frameshift?	Codons Altered		No. of TM motifs[b]	Phenotype	Ref.
					First	Stop			
1	Afg/Afg	ΔI55/ΔI55	4,4	No, No	—	—	2,2[c]	Very mild	9
2	Ita/Ger/ Ukr	E133X/T322insC	5,11	No, Yes	133,325	133,452	1,1	Mild	8
3	Jap/Jap	IVS5,+5G−>A/ IVS5,+5G−>A	—	Splicing	—	—	—	Typical	9
4	Ukr/Ukr	G319delG/S396delC	11,13	Yes, Yes	321,397	330,398	1,2	Typical	9
5	Jap/Jap	T322delC/T322delC	11,11	Yes, Yes	325,325	330,330	1,1	Severe	9
6	Swi/Swi	T322insC/T322insC	11,11	Yes, Yes	325,325	452,452	1,1	Mild	4,9
	Iri/Ger							Mild	4,9
	Fre/Fre							Typical	9
7	Dut/Ger	T322delC/S396delC	11,13	Yes, Yes	325,397	330,398	1,2	Mild	8
8	Sco/Sco	T322insC E666X	11,20	Yes, No	325,666	452,666	1,2	Mild	9
9	Ita/Eng/Iri/ Fre/Nor	S396delC/(S396delC)[d]	13,13	Yes, Yes	397,397	398,398	2,2	Severe	8,9
10	Jap/Jap	A441insA/A441insA	13,13	Yes, Yes	442,442	452,452	2,2	Typical	4,9
	PR/PR	16 bp dup/ 16 bp dup	15,15	Yes, Yes	497,497	587,587	2,2	Severe	4,8

[a] Afg, Afghan; Ita, Italian; Ger, German; Ukr, Ukrainian; Jap, Japanese; Swi, Swiss; Iri, Irish; Fre, French; Dut, Dutch; Sco, Scottish; Eng, English.
[b] Number of transmembrane (TM) regions remaining in the predicted, mutant peptide.
[c] The carboxy terminus remains intact in this mutation.
[d] This patient has been described as homozygous for the deletion (9), but hemizygosity cannot be ruled out (8).

mutant peptides which do not contain the transmembrane regions cannot integrate into membranes, so they cause milder phenotypes. Indeed, except for one Japanese patient (case 5) who is homozygous for T322delC, all groups of patients having amino acid sequence changes proximal to the second putative transmembrane domain in at least one mutant allele (e.g., cases 2, 4, 6, 7, and 8) have a mild or typical phenotype. In contrast, patient groups with amino acid changes distal to the second transmembrane domain (e.g., cases 9, 10, and 11) manifest a typical to severe phenotype. Moreover, an infant (case 1, Table 1) with one codon deleted from the *HPS1* gene but with an intact carboxy terminus has a mild phenotype (9).

Caution regarding this proposal remains warranted. First, there are relatively few mutations available to test the hypothesis. Second, even though the *HPS1* gene product has been proposed to be a transmembrane protein, no direct evidence has confirmed this hypothesis. Finally, disease severity must be judged by the overall phenotype, not simply by the degree of hypopigmentation (13), and we do not know for certain that this was done in all cases.

Mutation Detection

For the 16-bp duplication in exon 15 of *HPS1*, detection using PCR amplification is straightforward (4). A mutant product of 285 bp is easily distinguishable from the normal 269 bp band on a 2% agarose gel (13). This technique permits both prenatal diagnosis and carrier testing for HPS in the northwest Puerto Rican population. In fact, demonstration of homozygosity for the 16-bp duplication may supplant electron microscopic evidence of absent platelet-dense bodies as the diagnostic method of choice for patients of northwest Puerto Rican extraction. Note that HPS patients from other parts of Puerto Rico may have no *HPS1* mutation whatsoever (7), and molecular diagnosis would not be appropriate for at-risk individuals in this group.

Northern blot analysis can also help point to a mutation in *HPS1*. Case 2 in Table 1 had a decreased amount of normally sized *HPS1* RNA, and cases 7, 9, and 11 had no *HPS1* RNA by Northern blot analysis (8). In fact, every known *HPS1* mutation has yielded little or no normally sized mRNA. Of course, this technique will miss missense and other mutations, so screening with other methods

TABLE 2
Murine Models of Pigment Dilution and Platelet Storage Pool Deficiency[a]

Pigment mutation	Human chromosome	Mouse chromosome	Comment
1. pallid (*pa*)	15q11–ter	2	Greatest pigment dilution, lung emphysema, balance defect, enhanced response to morphine
2. cocoa (*coa*)	8q13–q22	3	No lysosomal dysfunction
3. subtle gray (*sut*)	3q24–q28	3	Mild pigment dilution, intermediate bleeding time, only serotonin reduced in dense granules, no lysosome dysfunction
4. light ear (*le*)	4p16	5	Large melanin granules in choroid, small eumelanin granules in hair
5. cappuccino (*cno*)	?	5	Elevated liver lysosomal enzymes
6. reduced pigment (*rp*)	19q13	7	Cutaneous hypopigmentation only, increased anesthetic susceptibility
7. ruby eye-2 (*ru-2*)	11p15/15q11	7	
8. mocha (*mh*)	12q22–q24.2	10	Small, sparse melanosomes in hair, hyperactivity, balance defect, δ subunit of AP-3 is mutated
9. muted (*mu*)	6p21–23/5p15	13	Balance defect
10. sandy (*sdy*)	6p21–23/5p15	13	
11. pearl (*pe*)	5q11–q14	13	Night blindness, β3A subunit of AP-3 is mutated
12. gunmetal (*gm*)	14q11.2–q13	14	Cutaneous hypopigmentation only, dense and alpha granule defect, abnormal expression of GTP-binding protein
13. pale ear (*ep*)	10q24.1–q25.1	19	Small eumelanin granules of hair, homologue of *HPS1*
14. ruby eye (*ru*)	10q24.1–q25.1	19	Reduced number of melanocytes

[a] Modified from Swank *et al.*, Ref. (38).

such as single-stranded conformational polymorphism (SSCP) analysis should be considered. Intragenic linkage analysis can also be useful in certain informative families.

Evidence for Locus Heterogeneity

It has become apparent that *HPS1* is not the only gene whose mutations result in the disorder HPS. Only 3 of 18 (8) and 10 of 24 (9) unrelated non-Puerto Rican HPS families were found by SSCP and/or heteroduplex analysis to have mutations in *HPS1*. Moreover, two individuals from central Puerto Rico, whose clinical diagnosis of HPS was confirmed by electron microscopy showing absent platelet-dense bodies, had no mutations upon sequencing of their entire *HPS1* cDNA; they also exhibited significant amounts of normally sized *HPS1* mRNA on Northern blot analysis (7). This indicates locus heterogeneity even within the Puerto Rican population, and the findings of Oh *et al.* (9) have supported this concept. Using homozygosity analysis of four consanguineous HPS patients with no mutations in *HPS1*, this group reported that three of the four were heterozygous for polymorphic markers flanking the *HPS1* gene on both sides. These findings suggest that several genes are individually responsible for the HPS phenotype, which is why we refer to *HPS* (4) as *HPS1*. Clearly, locus heterogeneity in humans is consistent with the existence of several mouse models of HPS manifesting both pigment dilution and a platelet storage pool deficiency.

Animal Models

At least 80 murine mutations have an effect on mouse coat color (36). Fourteen of these display a platelet storage pool deficiency and most manifest lysosomal defects (37,38). Consequently, these mutations serve as models of HPS (Table 2). All are inherited in an autosomal recessive manner, and mapping and complementation studies have documented that they represent different genes. Some of the murine mutants, such as *pallid* (39), are among the oldest identified mouse pigment phenotypes, while others, e.g., *cappuccino* (40), have only recently been described.

Although each mutant murine gene causes a phenotype related to HPS, the models are distinct. For example, *gunmetal* and *reduced pigment* have only cutaneous pigment dilution, while other mutant strains display oculocutaneous albinism (Table 2). There exists a rough correlation between the degree of albinism and the bleeding time in these mice. To date, three of the fourteen genes have been isolated and sequenced, i.e., *pale ear* (5,6), *pearl* (41), and *mocha* (42). Only for *pale ear* has a specific human

mutant phenotype (i.e., classical HPS) been described, although we know of a human pearl.

The mouse models can help solve the mysteries of human HPS in a variety of ways. They will assist in characterizing other human HPS genes by virtue of the syntenic relationship between murine and human genomes. Conversely, the mapping of human HPS genes can help identify genes responsible for the murine models. The mice can help to delineate the different phenotypes and natural histories associated with the various HPS-causing genes, and allow for investigation of the physiologic function of these genes in intact animals. Finally, the mutant mice will facilitate development of the most effective therapeutic interventions for the pathology of HPS. For example, bleeding tendencies and abnormally low platelet-dense granule contents have been corrected in several mouse mutants by bone marrow transplantation with normal cells, indicating that the defect occurs in marrow platelet progenitor cells. These findings also suggest that bone marrow transplantation may have a role in the treatment of HPS patients with severe bleeding problems. Moreover, the *pale ear* mutant exhibits pulmonary fibrosis (M. Brantly, personal communication, March 11, 1998), making it the first natural animal model with this manifestation. This mouse may help to determine whether a particular medication is safe and effective in preventing or treating the pulmonary fibrosis of HPS.

Vesicular Trafficking

Various lines of evidence suggest that improper formation of intracellular vesicles comprises the basic defect in HPS. First, three distinct organelles in different cell types, i.e., the melanosome in melanocytes, the dense body in platelets, and, most likely, the lysosome in other cells, manifest abnormalities. Second, those three intracellular organelles share at least one integral membrane protein. In the platelet, it is called CD63 or granulophysin (43); in the melanosome, ME491 (44); and in the lysosome, limp-1 or lamp-3 (45). Finally, two mouse models of HPS, *pearl* and *mocha,* have defects in subunits of a protein complex responsible for vesicle formation, i.e., the β3A (41) and δ (42) subunits of the AP-3 complex, respectively (Fig. 2). This provides clear precedent for abnormal vesicular trafficking machinery resulting in a combination of hypopigmentation and platelet storage pool deficiency. Incidentally, a *Drosophila* mutant called *garnet* also has a mutation in the δ subunit of AP-3 and presents with reduced pigmentation (46).

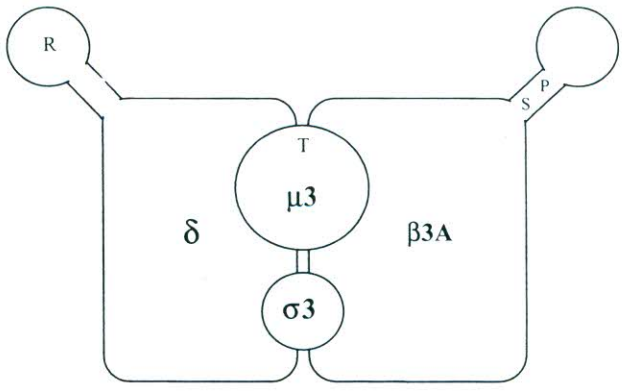

FIG. 2. Putative structure of the AP-3 adaptor complex. The complex consists of four subunits: δ-adaptin (160 kDa), β3A-adaptin (140 kDa), μ3A (47 kDa) and σ3A (22 kDa). β3A-adaptin consists of an amino-terminal domain (the core), a hinge region, and a carboxy-terminal domain (the ear). β3A-adaptin is thought to be highly phosphorylated (P) at serine residues of its hinge region; other subunits are not detectably phosphorylated. The hinge region of β3A-adaptin is also speculated to mediate interactions with scaffold proteins (S). The ear domain of δ-adaptin (R) may bind regulatory molecules such as dynamin. The μ3 chain is known to interact with tyrosine-based sorting signals (T). Other AP-3 adaptor complex subunits include β-NAP, a brain-specific variant of β3A-adaptin, μ3B, a brain-specific variant of μ3A, and σ3B, a variant of σ3A.

Protein trafficking and vesicle formation are intimately linked within eukaryotic cells, and the individual steps which compose these processes require a myriad of functional gene products. In pursuing the basic defect in HPS, it appears appropriate to examine how intracellular vesicles are formed, with an eye toward determining which of the requisite proteins might be dysfunctional in HPS.

One of the earliest steps in vesicle formation and protein sorting is cargo packaging. When the cargo consists of integral membrane proteins, the cytoplasmic tails of these proteins, containing tyrosine-, diphenylalanine-, dileucine-, and dilysine-based markers, interact with adaptor complexes such as AP-1 (47). Soluble cargo may need to bind first to an integral membrane protein (i.e., a membrane receptor) which subsequently binds to an adaptor complex or to a coatamer such as COPI or COPII. In this case, the proposed membrane receptor, such as the Emp24 group or the family of lectin-like proteins, should recognize targeting motifs shared by cargo destined for certain locations.

In HPS, the binding of cargo to adaptor complexes or other membrane receptors may be impaired, be-

cause those membrane receptors fail to recognize the cargo. This may be the defect in any of the genes causing HPS, including *HPS1*. Having a dileucine signal, the *HPS1* protein could bind directly to AP-3 (probably through the μ subunit), since it has been shown that dileucine-based signals interact with AP-2 (48). Undelivered cargo could result in the failure of melanogenic proteins in premelanosomes to arrive in melanosomes; this could cause hypopigmentation. Similarly, dense bodies may never receive their proper contents, and thus never become dense or recognizable or functional.

Another early step in organellogenesis is formation and budding of transport vesicles. A number of relatively small molecules, including the small GTPases such as ARFs (ADP-ribosylation factors) (47), phosphatidylinositides (49), and caveolin (50), facilitate and regulate this process, which involves recruitment of cytosolic coat proteins to the donor membrane. Coat protein complexes provide the structural components necessary to drive vesicle budding; they also impart cargo specificity by recognizing and packaging signal-bearing membrane proteins into the emerging vesicle. The first coated vesicles to be described were the clathrin-coated vesicles (51). Adaptor protein (AP) complexes link the integral membrane cargo or a specific soluble cargo transmembrane receptor with coat proteins. Examples of adaptor protein complexes include AP-1, associated the the trans-Golgi complex, and AP-2, associated with the plasma membrane.

A third adaptor protein complex has also been identified. AP-3, like AP-1 and AP-2, is heterotetrameric and consists of δ, β3A, μ3A, and σ 3 subunits (46,52–54). It has recently been recognized that AP-3 also binds clathrin, so this structural protein probably serves as the scaffold in AP-3-mediated budding processes. Studies in yeast (55) have suggested that AP-3 functions in cargo-selective protein transport from the Golgi to the lysosome. Since the μ2 subunit of the AP-2 complex has the ability to bind a subset of tyrosine-based signals (56), it is considered that μ3 serves the same function in AP-3. The involvement of mutant β3A and δ subunits in mouse models of HPS marks these proteins as prime candidates for defects causing the human disorder. In addition, the μ and σ subunits may be impaired in other patients and may be mutated in other mouse models of HPS. Finally, mutations in any of the ancillary proteins which prime the donor membrane for vesicle formation and budding could cause HPS, but some specificity for affecting melanosomes and platelet-dense bodies would be required.

The final steps in new vesicle formation are docking and membrane fusion. In current models, these processes are mediated by membrane proteins called v (for vesicle)-SNAREs and t (for target membrane)-SNAREs (57). A SNARE (SNAP, or soluble *N*-ethylmaleimide-sensitive factor attachment protein, receptor) is a vesicle trafficking protein whose hallmark characteristics include small size (15–40 kDa) and a compartment-specific carboxy terminus. The SNARE amino terminal and central regions reside in the cytoplasm, and specific binding between v-SNAREs and t-SNAREs mediates docking. ATP hydrolysis by *N*-ethylmaleimide-sensitive factor (NSF) fixes the membrane in fusion, possibly by dissociating the SNARE complex (57). Several other proteins, such as members of the rab and Sec1 families (58), also contribute to the process of membrane targeting and fusion. Clearly, selected proteins in this complex, if mutated, could account for the cell biological findings characteristic of HPS. Specifically, dense bodies may never form, melanosomes may fail to mature past stage II, and ceroid lipofuscin may represent the degradation products of misrouted intracellular membranes.

Since vesicular trafficking occurs in all cells, we must explain the restriction of clinical manifestations in HPS to those related to melanocyte and platelet dysfunction. In fact, the vesicular trafficking proteins mutated in HPS may be absolutely required for normal melanosome and dense body formation, whereas they may perform a redundant function in cells other than the melanocyte and platelet. There is precedent for this situation in the case of X-linked choroideremia, in which choroid and retinal depigmentation are the only signs of dysfunction of the rab escort protein-1, i.e., REP-1 (59,60); in other tissues/cells, different proteins can readily substitute for rep-1.

Future Pursuits

Several investigations beckon the student of HPS. In the clinical arena, individuals with different types of HPS must be extensively described and followed longitudinally for the various complications of this disorder, with an eye toward genotype/phenotype correlations. Patients with a bleeding diathesis and a tendency toward infections are of special interest, as they may represent variants in the gamut of disease between HPS and Chediak-Hi-

gashi syndrome. The frequency of pulmonary and gastrointestinal involvement in larger populations of HPS patients should be determined, and controlled studies of therapeutic agents initiated. Currently, a randomized, placebo-controlled trial of the antifibrotic agent pirfenidone (61) is being pursued at the National Institutes of Health.

Newly diagnosed HPS patients should be tested for defects in the *HPS1* gene. Those lacking such a mutation are candidates for having HPS due to defects in other genes, such as the AP-3 subunits. Other possibilities should also be investigated. In consanguineous families, homozygosity testing can be performed for polymorphic markers in regions syntenic to the known locations of murine HPS-causing genes. The cloning of these mouse genes themselves can lead to isolation of their human homologues, which then become candidates for involvement in HPS patients. Determining the cell biological function of *HPS1* should assume a high priority. This can be pursued by metabolic labeling studies and by examining which proteins interact with the *HPS1* gene product using the two-hybrid yeast system of protein interaction (62). The subunits of AP-3, in particular, should be investigated.

The rainbow of genes whose mutations result in HPS should lead to an understanding of the mechanisms of melanosomal and platelet-dense body organellogenesis; no matter what the frequency, each genetic defect will make its own contribution to the spectrum.

ACKNOWLEDGMENTS

The authors appreciate the cooperation of Mrs. Donna Appel and Dr. Carmelo Almodovar, presidents of the HPS Network in the mainland United States and in Puerto Rico, respectively, and of all the patients who contributed to our knowledge of HPS.

REFERENCES

1. Hermansky F, Pudlak P. Albinism associated with hemorrhagic diathesis and unusual pigmented reticular cells in the bone marrow: Report of two cases with histochemical studies. *Blood* **14:**162–169, 1959.
2. Witkop CJ, Quevedo WC, Fitzpatrick TB, King RA. Albinism. In The Metabolic Basis of Inherited Disease (Scriver CR, Beaudet AL, Sly WS, Valle DL, Eds.) 6th ed. New York: McGraw-Hill, Vol 2, pp 2905–2947, 1989.
3. King RA, Hearing VJ, Creel DJ, Oetting WS. Albinism. In The Metabolic and Molecular Bases of Inherited Disease (Scriver CR, Beaudet AL, Sly WS, Valle DL, Eds.). 7th ed. New York: McGraw-Hill, Vol 3, pp 4353–4392, 1995.
4. Oh J, Bailin T, Fukai K, Feng GH, Ho L, Mao J-i, Frenk E, Tamura N, Spritz RA. Positional cloning of a gene for Hermansky-Pudlak syndrome, a disorder of cytoplasmic organelles. *Nature Genet* **14:**300–306, 1996.
5. Gardner JM, Wildenberg SC, Keiper NM, Novak EK, Rusiniak ME, Swank RT, Puri N, Finger JN, Hagiwara N, Lehman AL, Gales TL, Bayer ME, King RA, Brilliant MH. The mouse pale ear (*ep*) mutation is the homologue of human Hermansky-Pudlak syndrome. *Proc Natl Acad Sci USA* **94:**9238–9243, 1997.
6. Feng GH, Bailin T, Oh J, Spritz RA. Mouse pale ear (*ep*) is homologous to human Hermansky-Pudlak syndrome and contains a rare 'AT-AC' intron. *Hum Mol Genet* **6:**793–979, 1997.
7. Hazelwood S, Shotelersuk V, Wildenberg SC, Chen D, Iwata F, Kaiser-Kupfer MI, White JG, King RA, Gahl WA. Evidence for locus heterogeneity in Puerto Ricans with Hermansky-Pudlak syndrome. *Am J Hum Genet* **61:**1088–1094, 1997.
8. Shotelersuk V, Hazelwood S, Larson D, Iwata F, Kaiser-Kupfer MI, Kuehl E, Bernardini I, Gahl WA. Three new mutations in a gene causing Hermansky-Pudlak syndrome: Clinical correlations. *Mol Gen Metab* **64:** 99–107, 1998.
9. Oh J, Ho L, Ala-Mello S, Amato D, Armstrong L, Bellucci S, Carakushansky G, Ellis JP, Fong C-T, Green JS, Heon E, Legius E, Levin AV, Nieuwenhuis HK, Pinckers A, Tamura N, Whiteford ML, Yamasaki H, Spritz RA. Mutation analysis of patients with Hermansky-Pudlak syndrome: A frameshift hot spot in the *HPS* gene and apparent locus heterogeneity. *Am J Hum Genet* **62:**593–598, 1998.
10. Witkop CJ, Babcock MN, Rao GHR, Gaudier F, Summers CG, Shanahan F, Harmon KR, Townsend DW, Sedano HO, King RA, Cal SX, White JG. Albinism and Hermansky-Pudlak syndrome in Puerto Rico. *Bol Asoc Med P Rico Agosto* **82:**333–339, 1990.
11. Lattion F, Schneider PH, DaPrada M, Lorez HP, Richards JG, Picotti GB, Frenck E. Syndrome d'Hermansky-Pudlak dans un village valaisan. *Helv Paediat Acta* **38:**495–512, 1983.
12. Schallreuter KU, Frenk E, Wolfe LS, Witkop CJ, Wood JM. Hermansky-Pudlak Syndrome in a Swiss population. *Dermatology* **187:**248–256, 1993.
13. Gahl WA, Brantly M, Kaiser-Kupfer MI, Iwata F, Hazelwood S, Shotelersuk V, Duffy LF, Kuehl EM, Troendle J, Bernardini I. Genetic defects and clinical characteristics of patients with a form of oculocutaneous albinism (Hermansky-Pudlak syndrome). *N Engl J Med* **338:**1258–1264, 1998.
14. Simon JW, Adams RJ, Calhoun JH, Shapiro SS, Ingerman CM. Ophthalmic manifestations of the Hermansky-Pudlak syndrome (oculocutaneous albinism and hemorrhagic diathesis). *Am J Ophthalmol* **93:**71–77, 1982.
15. Summers CG, Knobloch WH, Witkop CJ, King RA. Hermansky-Pudlak Syndrome: Ophthalmic findings. *Ophthalmology* **95:**545–554, 1988.
16. Witkop CJ, Krumwiede M, Sedano H, White JG. Reliability of absent platelet dense bodies as a diagnostic criterion for Hermansky-Pudlak syndrome. *Am J Hematol* **26:**305–311, 1987.
17. Witkop CJ, Wolfe LS, Cal SX, White JG, Townsend D, Keenan KM. Elevated urinary dolichol excretion in the Her-

mansky-Pudlak syndorme: Indicator of lysosomal dysfunction. *Am J Med* **82:**463–470, 1987.

18. Witkop CJ Jr, White JG, Townsend D, Sedano HO, Cal SX, Babcock M, Krumwiede M, Keenan K, Love JE, Wolfe LS. Ceroid storage disease in Hermansky-Pudlak syndrome: Induction in animal models. In Lipofuscin—1987: State of the Art (Zs.-Nasy I, Ed.). Amsterdam: Elsevier, p 413, 1988.
19. Garay SM, Gardella JE, Fazzini EP, Goldring P.M. Hermansky-Pudlak Syndrome: Pulmonary manifestations of a ceroid storage disorder. *Am J Med* **66:**737–747, 1979.
20. Harmon KR, Witkop CJ, White JG, King RA, Peterson M, Moore D, Tashjian J, Marinelli WA, Bitterman PB. Pathogenesis of pulmonary fibrosis: Platelet-derived growth factor precedes structural alterations in the Hermansky-Pudlak syndrome. *J Lab Clin Med* **123:**617–627, 1994.
21. Takahashi A, Yokoyama T. Hermansky-Pudlak syndrome with special reference to lysosomal dysfunction: a case report and review of the literature. *Virchos Arch A Pathol Anat Histopathol* **402:**247–258, 1984.
22. Hostre P, Willems J, Devriendt J, Lamont H, van der Straeten M. Familial diffuse interstitial pulmonary fibrosis associated with oculocutaneous albinism: Report of two cases with a family study. *Scand J Respir Dis* **60:**128–134, 1979.
23. Davies BH, Tuddenham EGD. Familial pulmonary fibrosis associated with oculocutaneous albinism and platelet function defect: A new syndrome. *Q J Med* **45:**219–232, 1976.
24. Schinella RA, Greco MA, Cobert BL, Denmark LW, Cox RP. Hermansky-Pudlak syndrome with granulomatous colitis. *Ann Intern Med* **92:**20–23, 1980.
25. Mahadeo R, Markowitz J, Fisher S, Daum F. Hermansky-Pudlak syndrome with granulomatous colitis in children. *J Pediatr* **118:**904–906, 1991.
26. Shanahan F, Randolph L, King R, Oseas R, Brogan M, Witkop C, Rotter J, Targan. Hermansky-Pudlak syndrome: An immunologic assessment of 15 cases. *Am J Med* **85:**823–828, 1988.
27. Van Dorp DB, Wijermans PW, Meire F, Vrensen G. The Hermansky-Pudlak syndrome: Variable reaction to 1-desamino-8D-arginine vasopressin for correction of the bleeding time. *Ophthal Paediatr Genet* **11:**237–244, 1990.
28. Wildenberg SC, Oetting WS, Almodovar C, Krumwiede M, White JG, King RA. A gene causing Hermansky-Pudlak syndrome in a Puerto Rican population maps to chromosome 10q2. *Am J Hum Genet* **57:**755–765, 1995.
29. Fukai K, Oh J, Frenk E, Almodovar C, Spritz RA. Linkage disequilibrium mapping of the gene for Hermansky-Pudlak syndrome to chromosome 10q23.1-23.3. *Hum Mol Genet* **4:**1665–1669, 1995.
30. Bailin T, Oh J, Feng GH, Fukai K, Spritz RA. Organization and nucleotide sequence of the human Hermansky-Pudlak syndrome (HPS) gene. *J Invest Dermatol* **108:**923–927, 1997.
31. Sharp PA, Burge CB. Classification of introns: U2-type or U12-type. *Cell* **91:**875–879, 1997.
32. Wildenberg SC, Fryer JP, Gardner JM, Oetting WS, Brilliant MH, King RA. Identification of a novel transcript produced by the gene responsible for the Hermansky-Pudlak syndrome in Puerto Rico. *J Invest Dermatol* **110:**777–781, 1998.
33. Barbosa MDFS, Nguyen QA, Tchemev VT, Ashley JA, Detter JC, Blaydes SM, Brandt SJ, Chotai D, Hodgman C, Solari RCE, Lovett M, Kingsmore SF. Identification of the homologous beige and Chediak-Higashi syndrome genes. *Nature* **382:**262–265, 1996.
34. Griffiths GM. Secretory lysosomes—A special mechanism of regulated secretion in haemopoietic cells. *Trends Cell Biol* **6:**329–332, 1996.
35. Ramsay M. Protein trafficking violations. *Nature Genet* **14:** 242–245, 1996.
36. Jackson IJ. Homologous pigmentation mutations in human, mouse and other model organisms. *Hum Mol Genet* **6:**1613–1624, 1997.
37. Novak EK, Hui S-W, Swank RT. Platelet storage pool deficiency in mouse pigment mutations associated with several distinct genetic loci. *Blood* **63:**536–544, 1984.
38. Swank RT, Novak EK, McGarry MP, Rusiniak ME, Feng L. Mouse models of Hemansky Pudlak syndrome: A review. *Pigment Cell Res* **11:** 60–80, 1998.
39. Roberts E. A new mutation in the house mouse (*Mus musculus*). *Science* **74:**569, 1931.
40. Peters LL, Gwynn B, Hunter SJ, Ciciotte SL, Eicher EM. Characterization and chromosomal localization of cappuccino, a new murine platelet storage pool deficiency (SPD) mutation. *Mol Biol Cell* **7:**146a, 1996.
41. Seymour AB, Feng L, Novak EK, Robinson MS, Swank RT, Gorin MB. A candidate gene for the mouse pearl (pe) mutation which affects subcellular organelles. *Mol Biol Cell* **8:**227A, 1997.
42. Kantheti P, Meyer GE, Carskadon SL, Gibson K, Kapfhamer D, Sufalko DS, Burmeister M. Mutations in an Adaptor-complex subunit explain the mouse mutant mocha. Implications for platelet storage pool deficiencies. *Am J Hum Genet* **61**S:A336, 1997.
43. Nishibori M, Cham B, McNicol A, Shalev A, Jain N, Gerrard JM. The protein CD63 is in platelet dense granules, is deficient in a patient with Hermansky-Pudlak Syndrome, and appears identical to granulophysin. *J Clin Invest* **91:**1775–1782, 1993.
44. Hotta H, Ross AH, Huebner K, Isobe M, Wendeborn S, Chao MV, Ricciardi RP, Tsujimoto Y, Croce CM, Koprowski H. Molecular cloning and characterization of an antigen associated with early stages of melanoma tumor progression. *Cancer Res* **48:**2955–2962, 1988.
45. Hunziker W, Geuze HJ. Intracellular trafficking of lysosomal membrane proteins. *BioEssays* **18:**379–389, 1996.
46. Simpson F, Peden AA, Christopoulou L, Robinson MS. Characterization of the adaptor-related protein complex, AP-3. *J Cell Biol* **137:**835–845, 1997.
47. Emr SC, Malhotra V. Membranes and sorting. *Curr Opin Cell Biol* **9:**475–476, 1997.
48. Heilker R, Manning-Krieg U, Zuber J-F, Spiess M. *In vitro* binding of clathrin adaptors to sorting signals correlates with endocytosis and basolateral sorting. *EMBO J* **15:**2893–2899, 1996.
49. Roth MG, Sternweis PC. The role of lipid signaling in con-

stitutive membrane traffic. *Curr Opin Cell Biol* **9:**519–526, 1997.

50. Harder T, Simons K. Caveolae, DIGs, and the dynamics of sphingolipid-cholesterol microdomains. *Curr Opin Cell Biol* **9:**534–542, 1997.
51. Goldstein JL, Anderson RGW, Brown MS. Coated pits, coated vesicles, and receptor-mediated endocytosis. *Nature* **279:**679–685, 1979.
52. Simpson F, Bright NA, West MA, Newman LS, Darnell RB, Robinson MS. A novel adaptor-related protein complex. *J Cell Biol* **133:**749–760, 1996.
53. Dell'Angelica EC, Ooi CE, Bonifacino JS. β3A-adaptin, a subunit of the adaptor-like complex AP-3. *J Biol Chem* **272:** 15078–15084, 1997.
54. Dell'Angelica EC, Ohno H, Ooi CE, Rabinovich E, Roche KW, Bonifacino JS. AP-3: an adaptor-like protein complex with ubiquitous expression. *EMBO J* **16:**917–928, 1997.
55. Cowles CR, Odorizzi G, Payne GS, Emr SD. The AP-3 adaptor complex is essential for cargo-selective transport to the yeast vacuole. *Cell* **91:**109–118, 1997.
56. Ohno H, Stewart J, Fournier M-C, Bosshart H, Rhee I, Miyatake S, Saito T, Gallusser A, Kirchhausen T, Bonifacino JS. Interaction of tyrosine-based sorting signals with clathrin-associated proteins. *Science* **269:**1872–1875, 1995.
57. Hay JC, Scheller RlH. SNAREs and NSF in targeted membrane fusion. *Curr Opin Cell Biol* **9:**505–512, 1997.
58. Novick P, Zerial M. The diversity of Rab proteins in vesicle transport. *Curr Opin Cell Biol* **9:**496–504, 1997.
59. van Bokhoven H, van den Hurk JAJM, Bogerd L, Philippe C, Gilgenkrantz S, de Jong P, Ropers HH, Cremers FPM. Cloning and characterization of the human choroideremia gene. *Hum Mol Genet* **3:**1041–1046, 1994.
60. Cremers FPM, Armstrong SA, Seabra MC, Brown MS, Goldstein JL. REP-2, a Rab escort protein encoded by the choroideremia-like gene. *J Biol Chem* **269:**2111–2117, 1994.
61. Iyer SN, Wild JS, Schiedt MJ, Hyde DM, Margolin SB, Giri SN. Dietary intake of pirfenidone ameliorates bleomycin-induced lung fibrosis in hamsters. *J Lab Clin Med* **125:**779–785, 1995.
62. Fields S, Song O-k. A novel genetic system to detect protein-protein interactions. *Nature* **340:**245–246, 1989.