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Commentary

Pigment, platelets, and Hermansky–Pudlak in human and mouse

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Although humans show a vast range of skin colors, our relative lack of hair prevents us from showing the full repertoire of pigment variation shared by most mammals (1). Nonetheless, we carry these genes and when their function affects more than pigment synthesis, hypomorphs may cause disease more complex than oculocutaneous albinism. These diseases sometimes have homologies in other species and, as mice are the mammal with the second-most advanced genetics (after humans), are frequently known in mice. The article by Gardner *et al.* (2) provides a good example of such a case which will allow *in vivo* studies of organelle function and intracellular movement. In other words, this cloning effort provides an animal model of a human genetic disorder affecting multiple functions.

Hermansky and Pudlak (3) were the first to describe patients with prolonged bleeding time and albinism. In the two unrelated cases, the albinism included the retina and was accompanied by congenital nystagmus. Histological studies found large reticular cells with an unusual pigment in the bone marrow of these patients. Although not a major topic of the report, chronic pulmonary disease, diagnosed as progressive pulmonary fibrosis in one patient and as a very poorly responsive tubercular process in the second patient, was also described. The cause of the prolonged bleeding time was not apparent by tests available at that time (3). The abnormal bleeding time was later determined to be due to a defect in platelet ADP release (4, 5). The material stored in the abnormal bone marrow cells has autofluorescent characteristics histochemically similar to that stored in neuronal ceroid-lipofuscinosis (6). Thus, Hermansky–Pudlak syndrome consists of a triad of albinism, platelets lacking dense bodies, and storage of an abnormal ceroid-like material in the reticuloendothelial system. These underlying abnormalities result in hypopigmentation, chronic bleeding problems, chronic interstitial pulmonary fibrosis, inflammatory bowel disease, kidney failure, and cardiac myopathy. The fundamental biochemical defect would seem to be one shared by membranes of melanosomes, lysosomes, and a derived lysosome, the platelet dense body. The striking similarity to the defects in pale ear mice suggested that these two were homologous (7).

Pale ear (*ep*) is a mouse mutation affecting coat pigmentation first described in 1967 (8). The homozygous recessive mice have decreased eye pigment at birth; as coat color appears, the amount of skin pigment is decreased compared with normal mice. Because this deficiency is most striking on the tail, feet, and the pinna of the ear, the mutation was named for the latter portion of the phenotype. Abnormal organelle function in these mice was described by Novak and Swank (9). Although the total rate of kidney protein synthesis and specific activities of several nonlysosomal enzymes were normal, pale ear mice showed several-fold increases in three renal lysosomal enzymes: β-glucuronidase, β-galactosidase, and α-mannosidase. This seemed to be due to a depression of secretion of enzyme into urine (9).

Abnormal secretion of lysosomal enzymes into urine was first described in the mouse model of Chediak–Higashi syndrome, the beige mouse. Chediak–Higashi syndrome is an autosomal recessive disorder with hypopigmentation, immunological deficiency, a bleeding tendency, and a neurological disorder (10, 11). A similar disorder has also been reported in mink (12) cattle (13), and cats (14). The disorder has most thoroughly been examined in mice (15, 16). In the case of the beige mouse, two groups independently reported the identification of the gene (17, 18). Surprisingly, however, the two cDNAs reported showed no significant matches. The discrepancy was resolved when the complete cDNA was obtained and it was discovered that one group had a 5′ portion and the other a 3′ portion of the same cDNA (19). The analysis of the predicted protein from this gene showed similarity to a yeast vacuolar sorting protein, VPS15 (19), demonstrating again that genetic and functional homology can extend far beyond classes or orders.

The gene for Hermansky–Pudlak syndrome was cloned using the positional cloning approach. This was greatly aided by use of the founder effect to perform homozygosity, also known as linkage disequilibrium, mapping (20). In this case, the very high prevalence in Puerto Rico, especially in the northwestern region of the island, and in a small village in the Swiss Alps, allowed the use of this method. In this approach, one takes advantage of the fact that if DNA is pooled from a number of individuals with the disorder in whom mutant alleles are shared by descent, and pooled DNA controls are used from relatives without the disorder, as microsatellites are examined across the genome they will show many bands in the pools of DNA until a chromosomal region where the patients must share alleles by descent is reached. In such a region, the microsatellite pattern will show a few shared bands among affected individuals and frequently can show only a single band if the marker is quite close to the gene. This approach was used in both the Puerto Rican (21, 22) and Swiss populations (22) where markers on distal chromosome 10 demonstrated such patterns. A number of markers in this region were then used in linkage studies and very high lod scores were found with several markers in the 10q23.1–q23.3 region.

Positional cloning of the human gene was recently reported (23). A yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), P1-derived artificial chromosome (PAC), and P1 contig was built across the region and cDNAs from a human melanoma library were selected with appropriate BAC and P1 clones bound to streptavidin-coated magnetic beads (23). A cDNA was found coding for a novel transmembrane protein and homozygous frameshift mutations in this gene were found in multiple patients (23). The Hermansky–Pudlak syndrome polypeptide is predicted to be a component of cytoplasmic organelles because of its transmembrane structure.

Gardner *et al.* (2) have cloned the pale ear and Hermansky–Pudlak genes on the basis of marked sequence conservation in the region of homology between the two species. Their starting

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point was human YACs which span the region of interest defined by human linkage studies. Subclones of the YACs were prepared and hybridized to human and mouse DNA. One small fragment was found which gave very strong cross-hybridization and which did not segregate from pale ear among over a 1,000 backcross mice. cDNA clones were obtained and one of these identified the same gene as that recently reported by Oh *et al.* (23). This mouse cDNA revealed multiple polymorphisms between the pale ear DNA and that from a congenic strain, suggesting that the mutation was associated with a major gene alteration. Indeed, sequencing of a mutant-specific DNA fragment showed that it contained sequences of an intracisternal A particle, a mouse transposon that is frequently found to be involved in mouse mutations (24). Sequence analysis of mutant RNA demonstrated that a chimeric mRNA would result with the loss of 46 C-terminal amino acids in the predicted pale ear protein and replacement by 78 intracisternal A particle-encoded amino acids (2). The importance of the gene product in most tissues was indicated by the finding that the cDNA for the pale ear gene was very widely expressed, although a very weak signal, and perhaps none, was found in skeletal muscle (2).

Gardner *et al.* (2) also provide further characterization of the pale ear mutation, documenting that its manifestations are highly homologous to those of Hermansky–Pudlak syndrome. Electron microscopy of ocular and skin melanocytes disclosed that they were about 15 times larger in volume than those in congenic controls. Collagen-mediated aggregation of platelets and release of ATP was very abnormal in platelets of pale ear homozygotes—findings very similar to those reported in patients with Hermansky–Pudlak syndrome (2).

The importance of this work is its demonstration of marked genetic and functional conservation between the human and mouse genomes in this chromosomal region. While some researchers have chosen to emphasize the few examples of differences between human and mouse genetic function (25), others have catalogued the many examples of marked homology between the two species (26, 27). In fact, some of the apparent differences are dependent on inbred strain background and are not due to basic difference in a homologous gene—i.e., the repertoire of modifying genes in some inbred strains in mice will be different than the usual modifying genes in human (28). The ability to map such modifiers in mice, and then use the well-described syntenic relationships to identify the homologous regions of the human genome, should provide a strong approach to mapping and cloning modifying genes in humans. For instance, a major modifier of the cystic fibrosis phenotype in mice (29) helped to identify a syntenic region in humans with a possible effect on the cystic fibrosis phenotype (30).

The importance of having mouse models is the opportunity they provide for studying the physiological function of mutant pathways in intact animals. This is particularly of use for studies attempting to modify the phenotype by therapeutic studies. Thus, mouse models can be used to test classical pharmacologic agents as well as to assess the value of gene therapy modalities. In addition, as the human genome and mouse genome projects identify thousands of new genes whose impact on physiological function are unknown, mouse models provide a unique opportunity to study the interactions of these genes. For instance, mice doubly homozygous for knockout mutations have allowed finer analysis of the importance of the genes. An example is the use of mice doubly homozygous for MyoD and myogenin or Myf-5 and myogenin to explore the role of these helix–loop–helix proteins in skeletal muscle differentiation (31). The results indicate that no more than two of the four myogenic factors are needed in this pathway. In the future, I believe that we will see many more physiological/phenotypic analyses of mice multiply mutant for both knockout and "naturally" occurring mutations. Such crosses will allow the determination of epistatic interactions and the ordering of genes in developmental pathways.

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