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The light side of platelet dense granules

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An article in this issue of *Blood* provides genetic and molecular insights into a critical but relatively little understood aspect of platelet physiology, that is, the biosynthesis of platelet dense granules.

Gwynn and colleagues use a positional cloning approach to identify the gene mutated in the reduced pigment *(rp)* mouse, a model for human Hermansky-Pudlak syndrome (HPS). HPS is a genetically heterogeneous disease whose symptoms (prolonged bleeding, light or hypopigmentation, and lung fibrosis) arise from defective biogenesis and/or trafficking of lysosome-related organelles including platelet dense granules, melanosomes, and lamellar bodies of lung type II cells, respectively.[1] The *rp* gene encodes a novel, widely expressed 195–amino acid protein. The novel nature of the *rp* gene is a recurring theme among 10 recently identified HPS genes.

All 10 novel HPS proteins interact within 3 distinct ubiquitously expressed protein complexes or biogenesis of lysosome-related organelle complexes (BLOCs). Gwynn and colleagues show that the *rp* protein coimmunoprecipitates with the pallidin, cappuccino, and muted HPS proteins, indicating that it is a component of the BLOC-1 complex, perhaps the best characterized of the 3 HPS BLOCs. Dell'Angelica et al[2] have independently identified the *rp* mutation and the residence of its encoded protein within BLOC-1. Additionally they have used proteomic approaches to define 3 additional BLOC-1 components, the novel proteins BLOC subunits 1 and 2 (BLOS1 and BLOS2) together with the coiled-coil protein, Snapin. Altogether then BLOC-1 contains 7 novel proteins, and mutations in at least 5 of them produce HPS in mice. The fact that the phenotypes of BLOC-1 mutants are the most severe of all mouse HPS mutants suggests that this complex plays a major role in the biogenesis of platelet dense granules and other specialized lysosome-related organelles of metazoans.

These exciting findings provide an entry for determining of the molecular mechanisms used by BLOCs to control the biosynthesis and intracellular movement of lysosome-related organelles. Missing or significant reductions in numbers of platelet dense granules is a hallmark of HPS in humans and animal models. Genes such as *rp* will therefore provide valuable insights into the synthesis and trafficking of this organelle, which is critical for normal platelet aggregation and function. One possible clue to the mechanism is the finding of Gwynn and colleagues that the *rp* protein in BLOC-1 is phosphorylated.

These and related findings give an in-depth view of the mutations and genes involved in the genesis of HPS that will hopefully lead to therapies for this inherited, presently incurable disease. ■

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A choline "vacuum cleaner"

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A powerful inward choline transporter on the plasma membrane of intraerythrocytic *Plasmodium falciparum (P falciparum)* parasites serves as an entry pathway for a promising family of new antimalarial agents.

Choline is used by malaria parasites developing within infected red blood cells (IRBCs) for the synthesis of phosphatidylcholine, an essential component of newly forming parasite membranes. Normal human red blood cells (RBCs) have a saturable carrier for choline uptake, but its maximal transport capacity is minute, far below parasite requirements. To ensure adequate supply from micromolar plasma levels, the parasite evolved a powerful transport and sink mechanism. The transport chain operates via 2 transporters in series, 1 on the host cell membrane and 1 on the parasite plasma membrane (PPM). Host membrane transport is entirely passive through a nonsaturable pathway, the well-characterized "new permeation pathway" (NPP), a broad-specificity anion channel. From the host, choline reaches the PPM transporter through the parasitophorous vacuolar membrane, assumed to present no permeability barrier for choline. Within the parasite, the free choline concentration is rapidly reduced by kinase-catalyzed choline phosphorylation, rate-limited by the choline supply through the PPM transporter.[1] This arrangement operates like a choline sink presumably by maintaining a steady inward choline gradient. In this transport and sink chain, the most serious gap in our knowledge concerns the properties of the PPM choline transporter. The PPM choline transporter has attracted major interest as a possible transport route for a new class of potent antimalarials for which choline, a quaternary ammonium monovalent cation, served as lead compound: bisamidine and bis-quaternary ammonium compounds.[2,3] Tested both in vitro and in vivo, these compounds proved to have potent activity against *Plasmodium falciparum (P falciparum),* but their access pathway to the parasite was unknown.

In this issue of *Blood,* Biagini and colleagues provide a thorough functional and kinetic characterization of choline transport across the parasite PPM and show that bisamidine and bis-quaternary ammonium compounds gain access to the parasite cytoplasm

via the PPM choline transporter. Using a preparation of *P falciparum* parasites freed from host permeability constraints by saponin permeabilization,[4] they showed that choline transport is carrier mediated, with a $K_{1/2}$ of about 25 μM and a maximum velocity (V_{max}) of 4.6 pmol $(10^6 \text{ cells})^{-1}\text{min}^{-1}$. They compared the rates of choline transport through NPPs and through the PPM carrier at physiologic choline concentrations and demonstrate that choline entry through NPPs is rate limiting. They conclude that in steady-state, the host choline concentration will be depleted relative to the extracellular medium and describe the PPM transporter as a "vacuum cleaner" of choline entering the host cell. Choline transport was inhibited competitively by pentamidine, a bis-cationic choline analog, but with a dissociation constant of an inhibitor (K_i) far above the antimalarial median effective concentration (EC_{50}), suggesting that inhibition of PPM choline transport may not be a significant contributor to the antimalarial effects of these compounds. Based on indirect evidence, Biagini and colleagues suggest that the PPM choline transporter is electrogenic, a conclusion firmly supported in recently published work by Lehane et al.[5] These authors showed that the PPM membrane potential can energize choline accumulation within the parasite when the phosphorylation sink is blocked by adenosine triphosphate (ATP) depletion. It may appear puzzling that choline uptake needs energizing when the metabolic sink is expected to maintain a steady inward gradient. The answer may be to ensure the internalization of essentially all the choline made available to the parasite by the potential-energized choline vacuum cleaner. ■

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