Coordinated trafficking of heme transporters by cargo sorting complexes is essential for organismal heme homeostasis

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Heme, an iron-containing organic ring, is a vital cofactor responsible for diverse biological functions and is the major source of bioavailable iron in the human diet. As a hydrophobic and cytotoxic cofactor, heme must be transported in a highly controlled manner through membranes via specific intra- and inter-cellular pathways. However, the genes and pathways responsible for heme trafficking remain poorly understood. Unlike other metazoans, *Caenorhabditis elegans* cannot synthesize heme but requires heme for sustenance. Thus, *C. elegans* is an ideal animal model to identify heme trafficking pathways as it permits organismal heme homeostasis to be directly manipulated by controlling environmental heme. Heme is imported apically into the intestine by HRG-1 transmembrane permeases and exported basolaterally by MRP-5/ABCC5 to extra-intestinal tissues. Loss of MRP-5 causes embryonic lethality that can be suppressed by dietary heme supplementation raising the possibility that MRP-5-independent heme export pathways must exist. Here we show, by performing a forward genetic screen in *mrp-5* null mutants, that loss of the vesicular cargo sorting Adaptor Protein complexes (AP-3) fully rescues *mrp-5* lethality and restores heme homeostasis. Remarkably, intestinal heme accumulation due to *mrp-5*-deficiency causes degradation of the lysosomal heme importer HRG-1. However, loss of both MRP-5 and AP-3 subunits resurrects HRG-1 levels and localization, thus underscoring the crucial role of HRG-1 in dictating *mrp-5* mutant phenotypes. In the absence of MRP-5, heme is exported by SLC49A3 homologs, a previously uncharacterized transporter. Live-cell imaging reveals vesicular coalescence that facilitates heme transfer between the importers and exporters at the interface of lysosomal-related organelles. These results define a mechanistic model for heme trafficking and identifies SLC49A3 as a heme exporter in metazoa.