Introduction

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Leishmaniasis is caused by the protozoan parasite *Leishmania* and affects several million people worldwide (Herwaldt, 1999). The disease is manifested in pathologies that range from mild cutaneous lesions to fatal visceral Leishmaniasis, the outcome determined in part by the species of *Leishmania* and in part by host factors that are associated with the innate and acquired immune responses. Kala-azar, a chronic and often fatal form of human visceral Leishmaniasis is mainly found in Mediterranean Europe, South America particularly Brazil, North and east Africa, India, China and is predominantly caused by *Leishmania donovani.*

During its life cycle, *Leishmania* exist either as intracellular amastigotes within a specialized phagolysosomes of vertebrate macrophages or as extracellular promastigotes in the digestive tract of their vector, the sand fly. *Leishmania* and its close relative *Trypanosoma* represent a highly divergent eukaryotic lineage having many unique features at the molecular and cellular level. Compared to the mammalian cells, the organization and function of different intracellular organelles, especially the molecular machinery underlying secretory and endocytic processes of protozoa have not been studied in details.

A number of receptor systems like those for LDL, transferrin and fibronectin have been identified on the surface of trypanosomatids (Overath *et al.*, 1997) presumably, to endocytose essential nutrients. Recent studies from our laboratory have shown that hemoglobin endocytosis in *Leishmania donovani* is mediated through a 46 kDa protein located in the flagellar pocket (Sengupta *et al.*, 1999) probably to generate intracellular heme, as these parasites are unable to synthesize heme (Sah *et al.*, 2002). However, the intracellular route and mechanisms of transport in parasites remain to be explored.

Since endocytosis is the major route of entry of nutrient into cells, understanding the membrane trafficking events in *Leishmania* and their modulation by other signal transduction intermediates may provide new insights into the extent to which the basic machinery is conserved throughout evolution. However, endocytic mechanisms are largely inaccessible to direct biochemical manipulation because the component parts are located mostly in the cytoplasmic side of the membrane. The classical biochemical approach to unravel cellular mechanisms involved in protein trafficking is to develop a cell free assay and progress has been made in understanding the

regulation of intracellular trafficking of endocytosed molecules in higher eukaryotic cells using similar assays (Gorvel *et al.*, 1991). During the past few years, it has been shown that Rabs, which are GTP binding proteins of Ras superfamily, are versatile molecular switches that regulate intracellular transport through vesicle fusion.

Previous results have shown that GTP binding proteins of Ras superfamily are well conserved among different species and perform similar functions. It is reasonable to assume the existence of similar proteins in *Leishmania,* which may regulate intracellular trafficking of endocytosed molecules. Moreover, our previous results have shown that following initial binding of hemoglobin with its putative receptor localized on the cell surface of *Leishmania* promastigotes, hemoglobin is rapidly internalized into discrete intracellular vesicles, which possibly indicates that endocytosis and intracellular trafficking in *Leishmania* are regulated by vesicle fusion and fission.

In the present investigation, we have developed an *in vitro* reconstitution assay of vesicle fusion to understand the mechanism of intracellular trafficking of hemoglobin in *Leishmania.* We have shown that hemoglobin first enters into an early compartment of *Leishmania* promastigotes and is subsequently transported to the late compartment and this intracellular trafficking is regulated by specific Rab GTPases, cloned from *Leishmania,* through vesicle fusion. Moreover, our results represent the first documentation that the route of hemoglobin trafficking inside *Leishmania* is directed by the signals generated from the hemoglobin receptor tail.