

Leishmaniasis



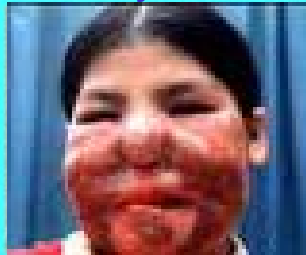
Sand Fly



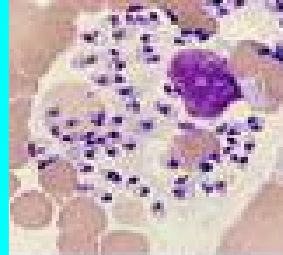
Cutaneous Leishmaniasis



L. tropica



Muco-cutaneous



Detection



PKDL



Visceral

L. brazillensis &
L. mexicana



L. Donovanii (Kala-azar)
& *L. infantum*

Detection of parasite from splenic aspiration or from bone marrow puncture.

Treatment:

Pentavalent antimonial like sodium stibogluconate, meglumine:

Very toxic sometimes with life-threatening adverse side effect like cardiac failure & acute pancreatitis

Amphotericin B:

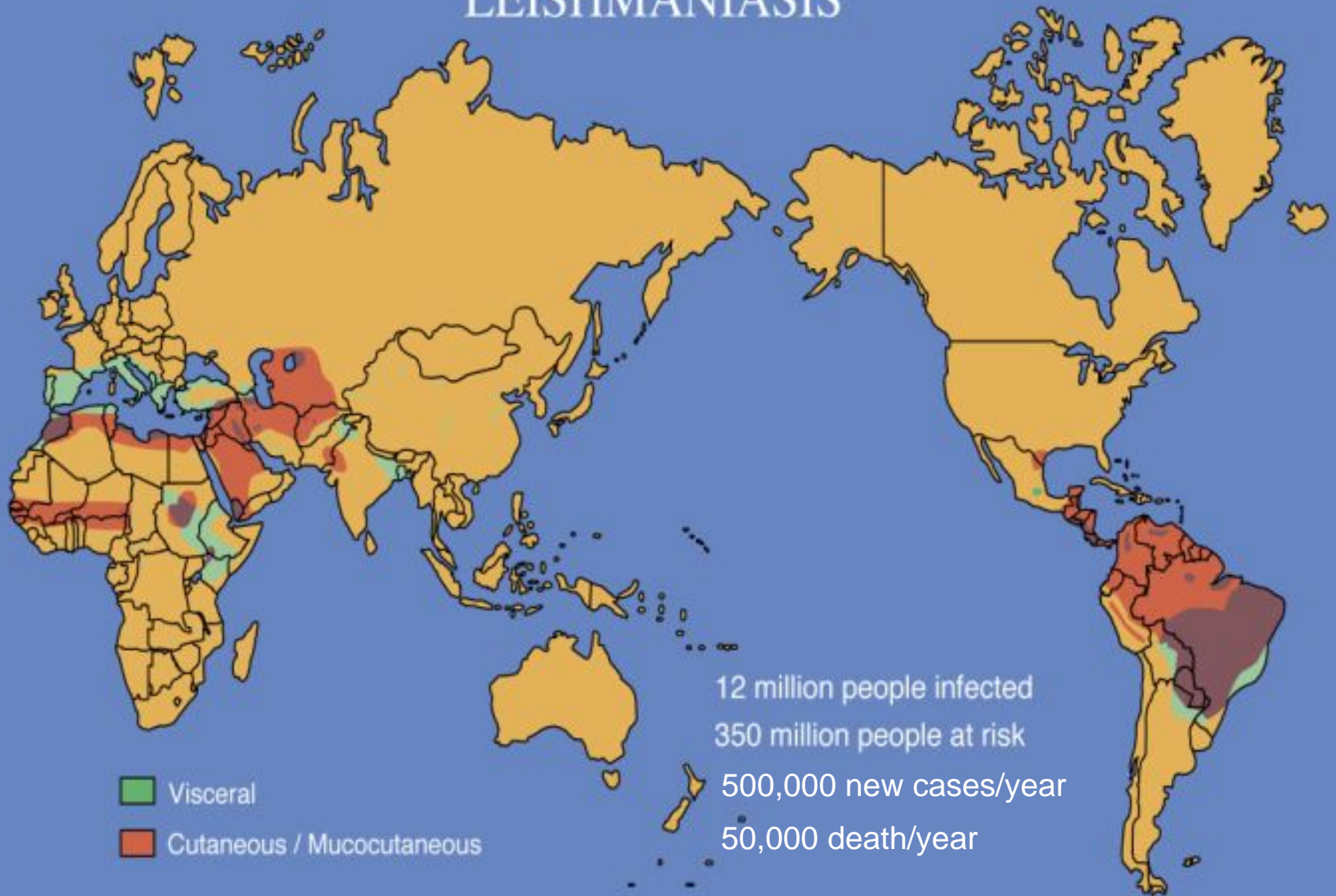
Costly also very toxic causes cardiotoxicity and nephrotoxicity, require hospitalization.

Miltefosine:

Originally anticancer drug used in VL, toxic and very costly.

No vaccine and appropriate diagnostic procedure are available against leishmaniasis.

LEISHMANIASIS



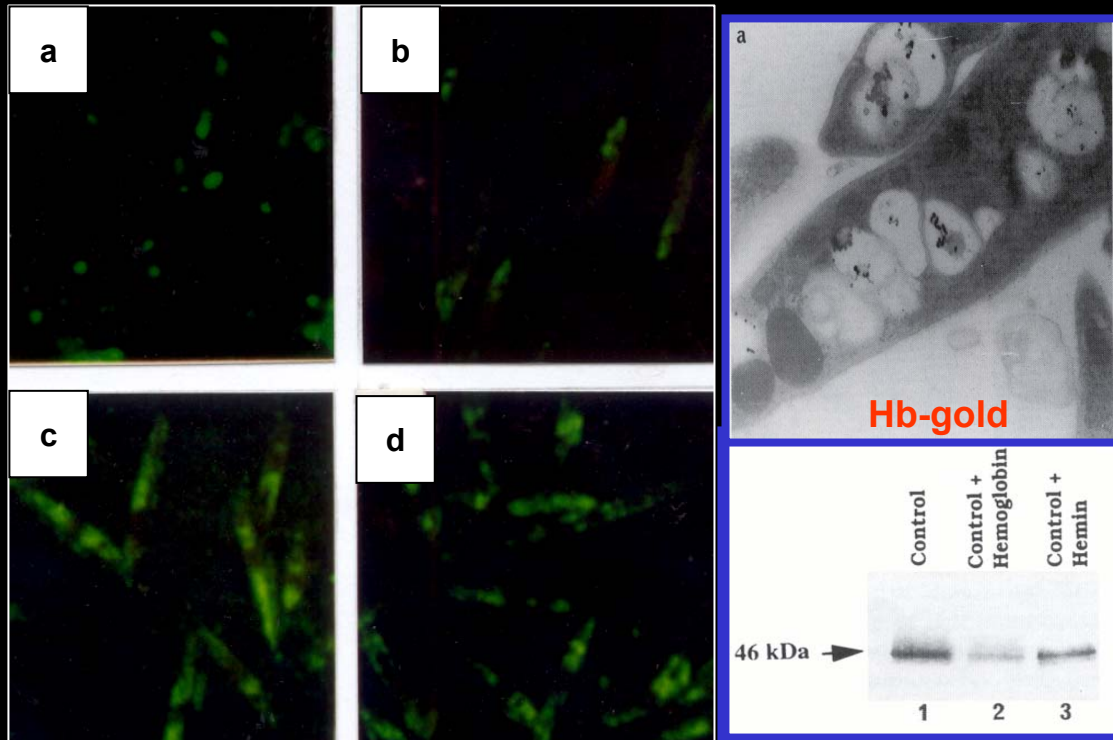
Leishmania lack a complete heme biosynthetic pathway and must acquire heme from external sources for their survival.

How they acquire heme?

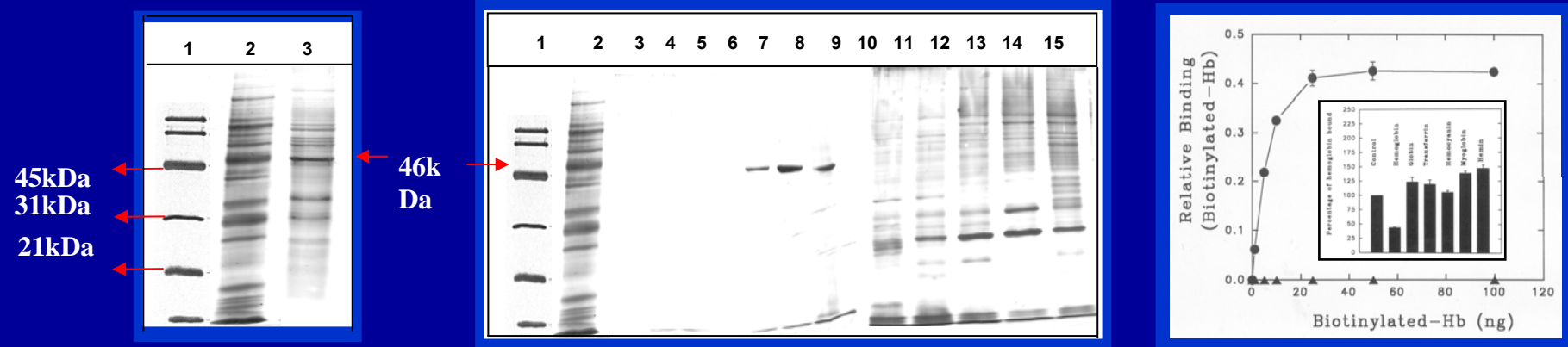
Is it from host hemoglobin?

Hemoglobin endocytosis in *Leishmania*: A novel target

46-kDa protein located in the flagellar pocket of *Leishmania* involved in hemoglobin endocytosis

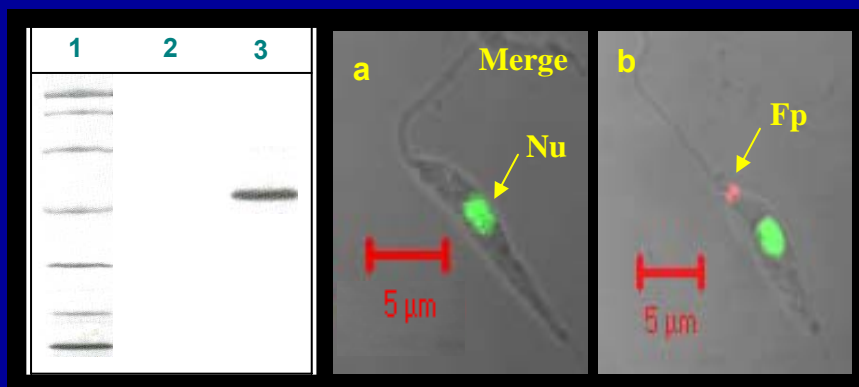


Purification and characterization of hemoglobin receptor from *Leishmania* promastigotes



• Membrane proteins from *Leishmania* promastigotes were solubilized in octyl β -glucoside and ~46 kDa protein was enriched by 50% ammonium sulfate precipitation. Pellet resuspended in low salt buffer containing Triton-X100 was loaded onto SP-Sepharose column. Protein was eluted along a linear gradient of salt concentration. The ~46 kDa receptor protein eluted in fractions corresponding to salt concentration of about 140-190 mM. Hb specifically binds with purified protein.

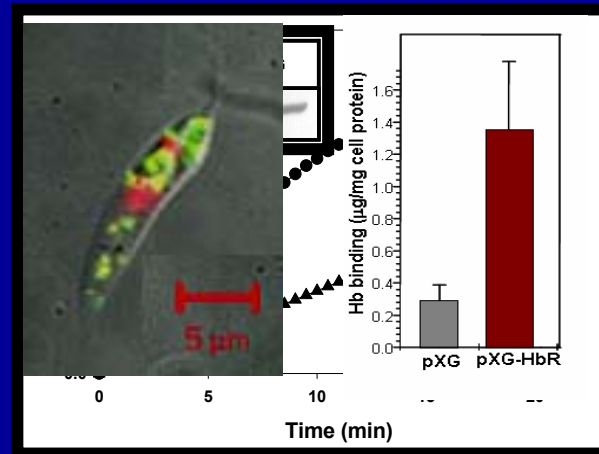
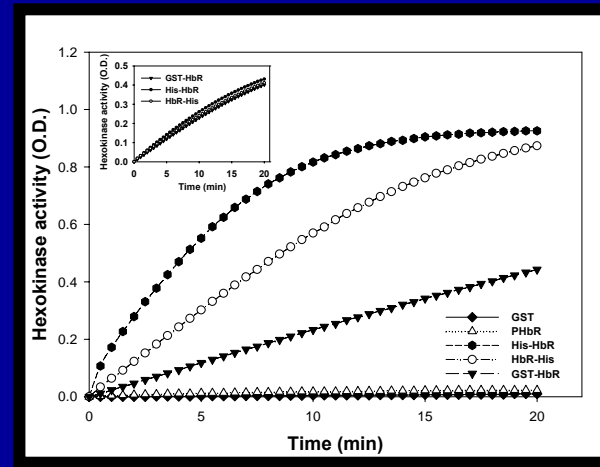
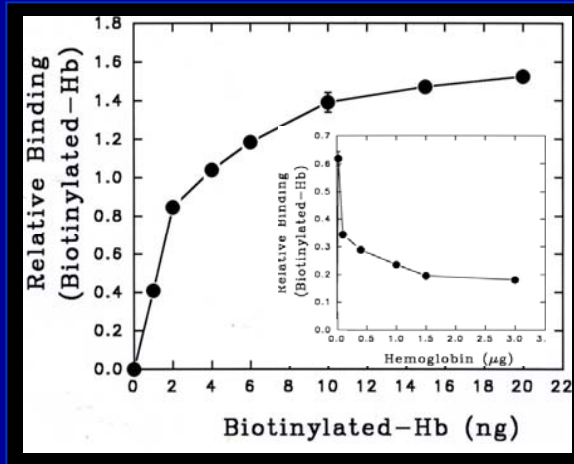
Localization of the 46-kDa protein in *Leishmania*



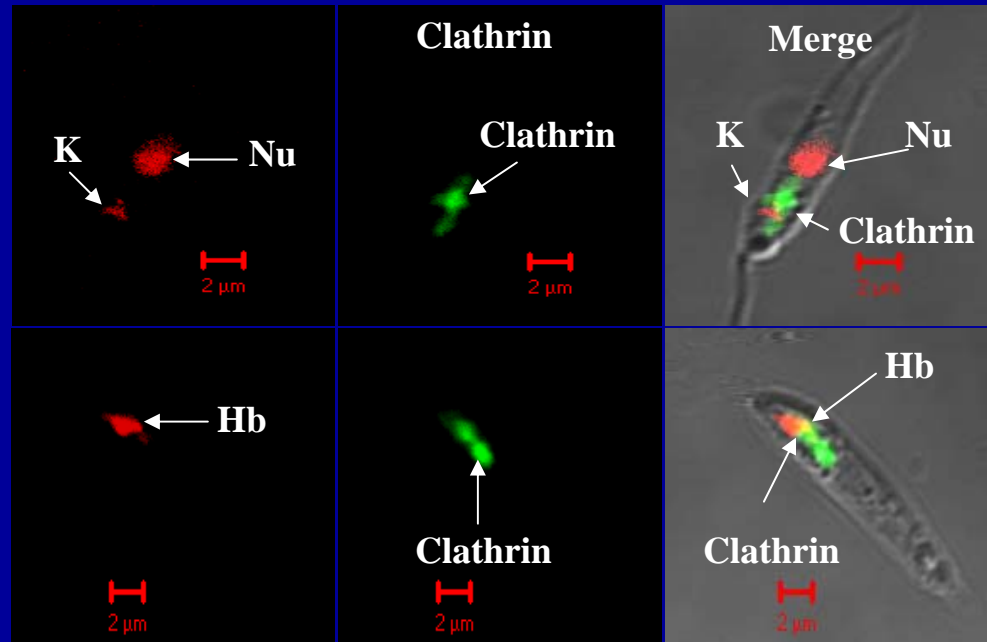
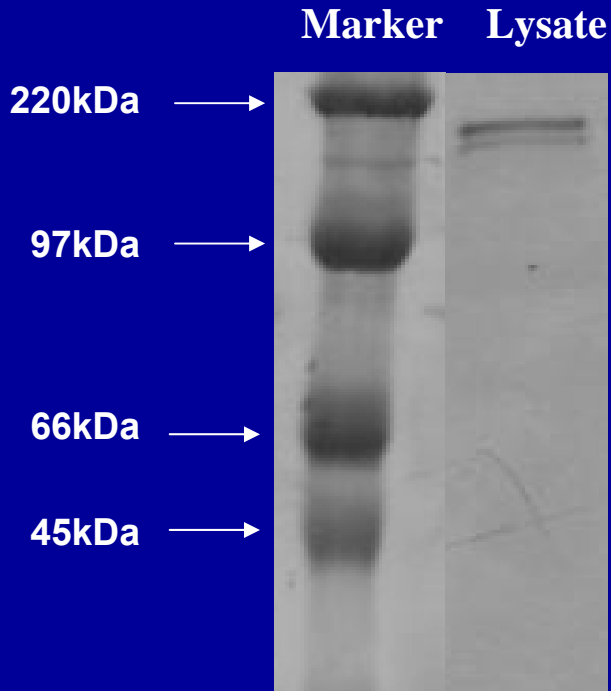
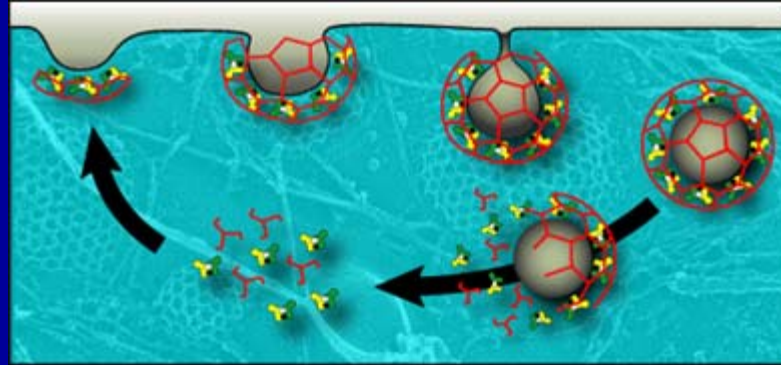
- | | |
|-------------------------|-------------------------------------|
| 1. GXXXK | 9. MXHEMVEGXEGR |
| 2. VVXQK | 10. MXPSFVYTSDA PK |
| 3. FVXPK | 11. MMSENAPDDXEK |
| 4. QNXQR | 12. TQGXATVAVDG SVYEK |
| 5. TPSFKR | 13. DGSGVGAAMX - - - K |
| 6. VNNXXSHXAXR | 14. GNAVTPXNMEC ^a GNFDSK |
| 7. ASAVTKDPAVSAR | 15. - - - GMXAAD - - - |
| 8. XVSGMYXSVXSR | 16. MMSENAPDDXEK |
| 17. SAXVGDATDXFDXQAQSVK | |

Blast search indicated that some of the peptides could partially aligned with full length hexokinase from different organisms.

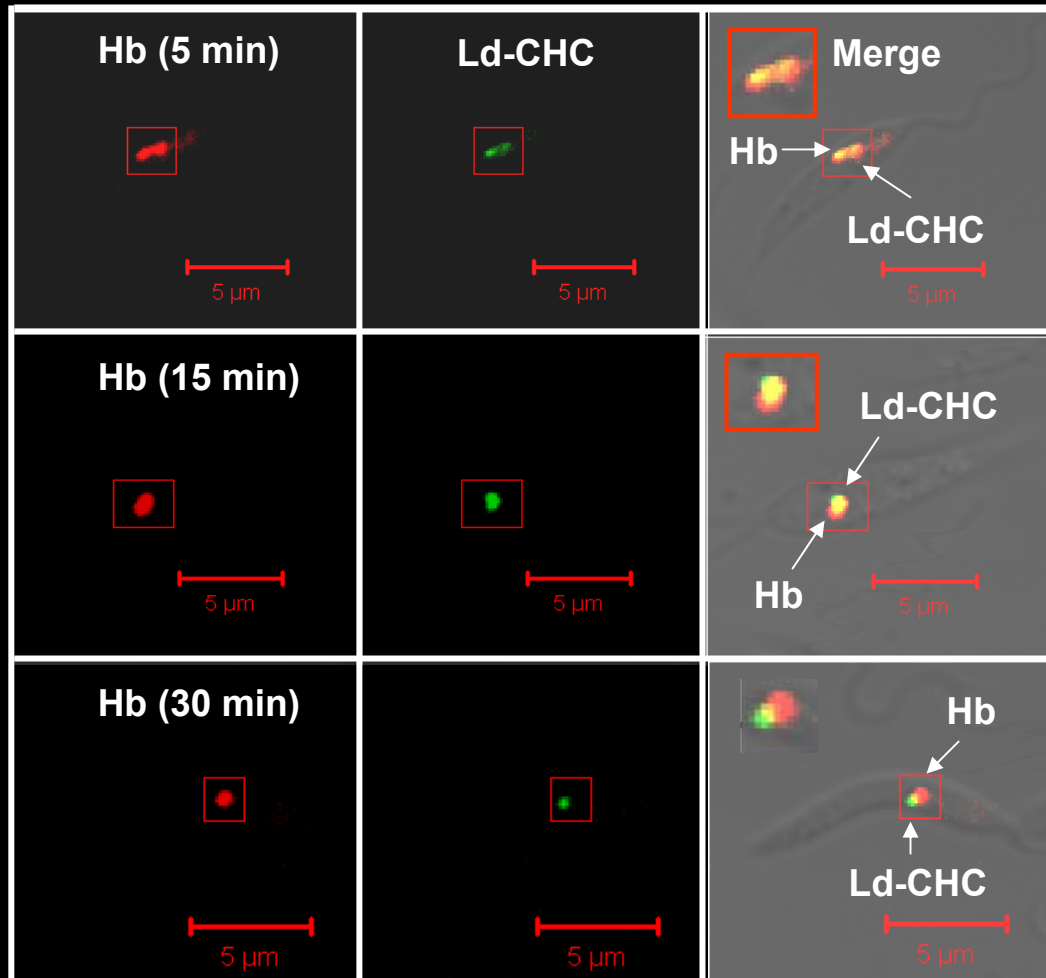
Hemoglobin receptor in *Leishmania* is a hexokinase



Role of clathrin and dynamin in hemoglobin endocytosis in *Leishmania*

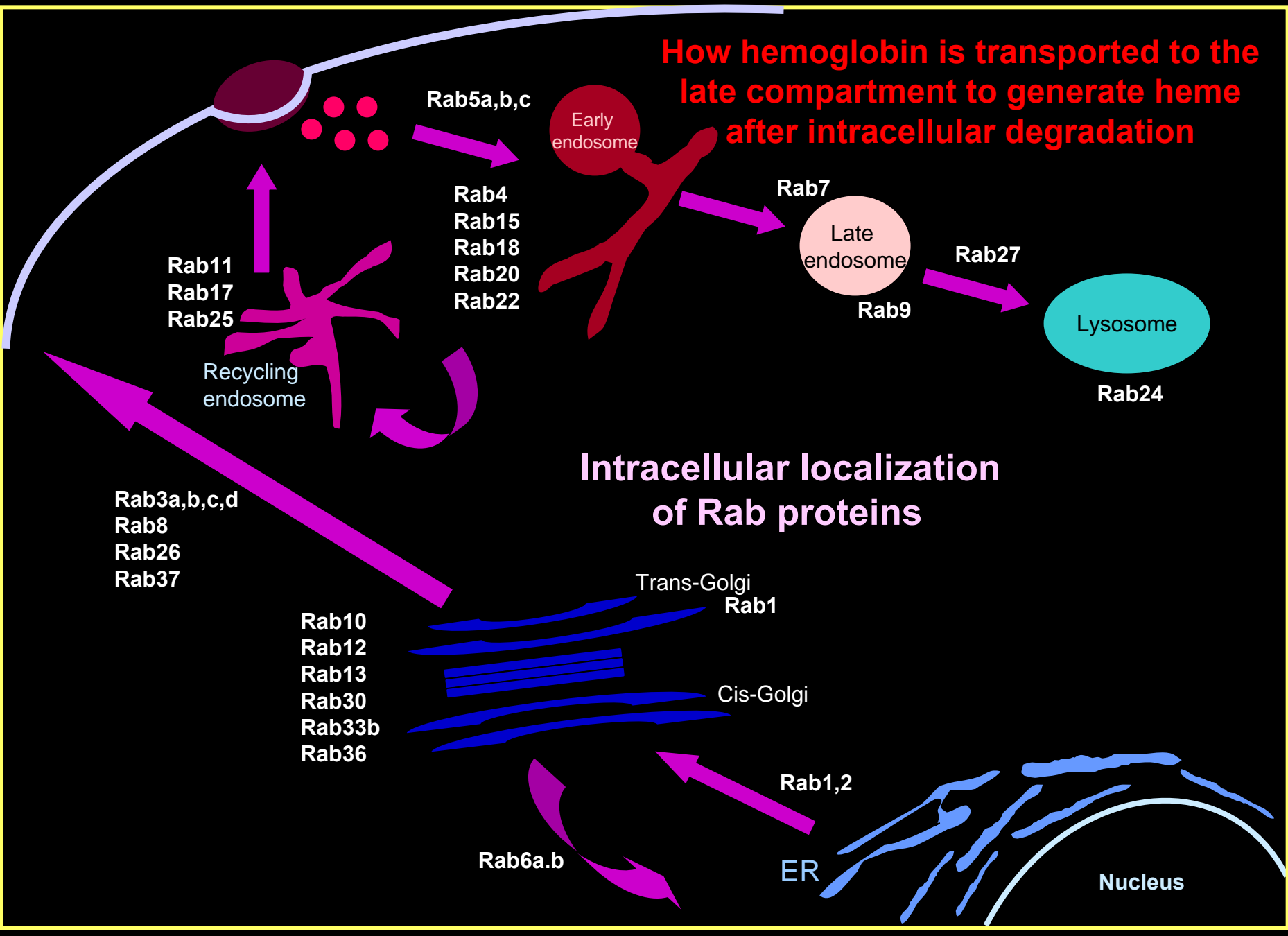


Role of clathrin in hemoglobin trafficking in *Leishmania*



How hemoglobin is transported to the lysosomess in *Leishmania*

How hemoglobin is transported to the late compartment to generate heme after intracellular degradation



Cloning and expression of endocytic Rab GTPases from *Leishmania*

Cloning and expression of Rab5 from *Leishmania donovani*

PCR Amplification of *Leishmania* Rab5 from cDNA



Forward 5'-GGATCCATGTCATCCATCAGTCGC-3'
Bam HI

Reverse 5'-GAATTCCTAGCAG CATCCGTTCTCT-3'
EcoRI

~650 bp

Putative Rab5 amino acid sequence (211 aa):

MSSISRATT TAGGSASTRK FKLVLGEGS VGKSSVVQRL MKNAFSEKLN
STVGASFFRY TCNVDDTAV HFDIWDTAGQ ERFKSLASMY YRGAAAALVV
FDIVSADTFE KARYWIRELQ ANSPETIVML VGNKKDLESE RQVSVADAQQ
CAVEMGAVYH ETSARSGDGV RDAFHAVAAK LIETNSAFSV REGGVMCHTE
NAAPRQENG C C*

(conserved domains of Rab proteins)

Cloning and expression of Rab7 from *Leishmania donovani*

PCR Amplification of *Leishmania* Rab7 from cDNA



Forward 5'-GGATCCATGTCGATGAAGCGGCA-3'
Bam HI

Reverse 5'-GAATTCCTAGCAGCTGCAGGCGG-3'
Eco RI

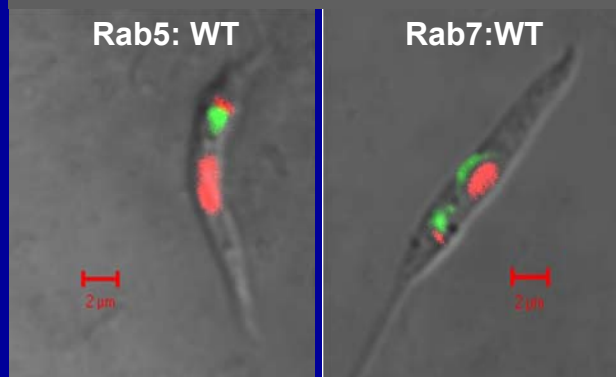
~700 bp

Putative Rab7 amino acid sequence (223 aa):

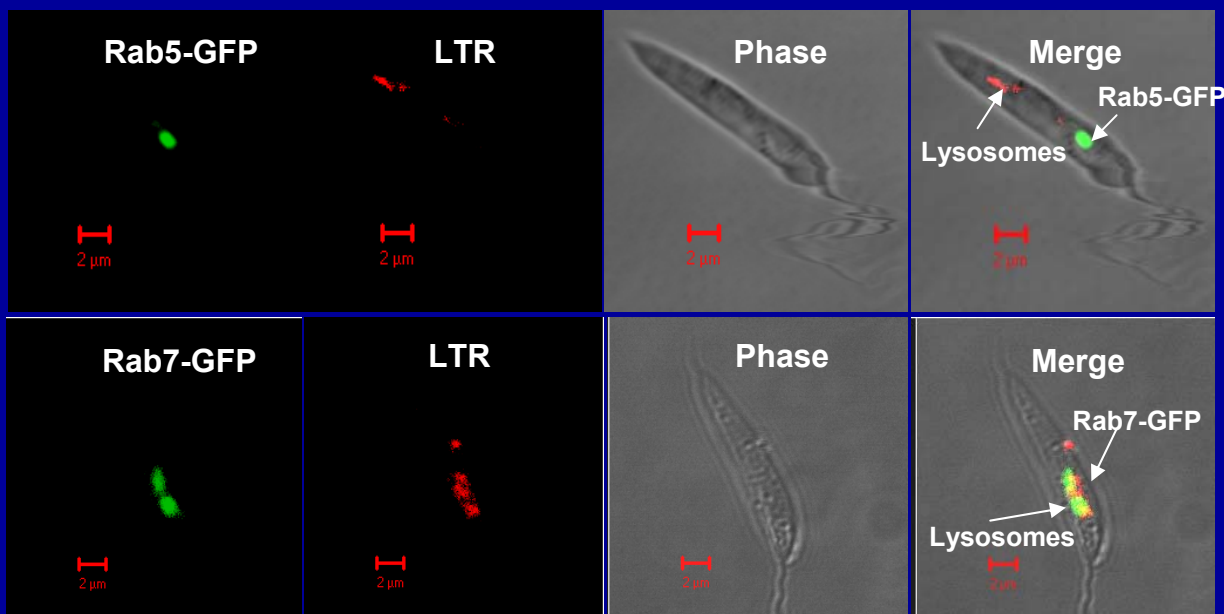
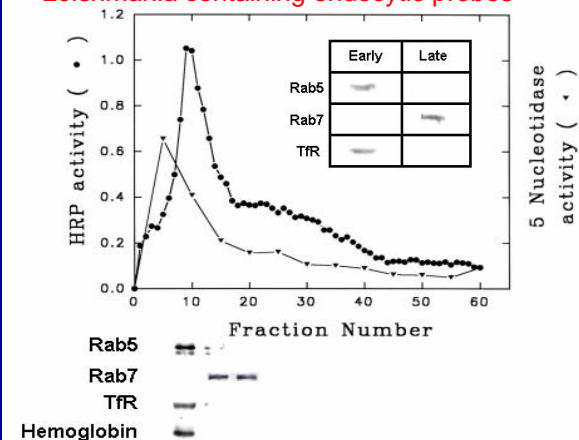
MSMKRQLLKI IILGDSGVGK TSLMHQYVNR IFDNRYKATI GADFLSKDVE
VNGCVVTLQI WDTAGQERFQ SLGSIFYRGA DACILVFDVT QQESFAHVG
WLEEFISIAG RRDSVLVGNK TLEDRRQVA SKTVQAWCAK QNAEAAANAIN
GACAGAGDSA APEMKYFETS AKDNAGVEEA FIAVVQLALA RKATVEEATP
MPQTVNLSQA QHEQTPTSSA CSC*

(conserved domains of Rab proteins)

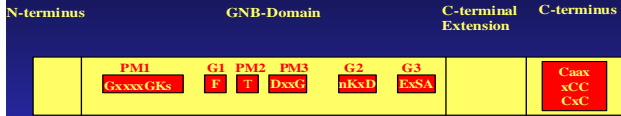
Localization of Rab5 and Rab7 in *Leishmania*



Fractionation of early compartment from *Leishmania* containing endocytic probes

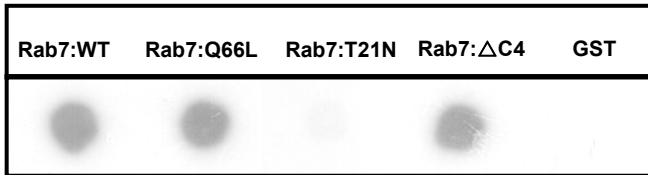


Main sequence elements and very conserved residues of ras-like proteins



X = any residue
 a = aliphatic
 s = Ser or Thr

GTP Binding

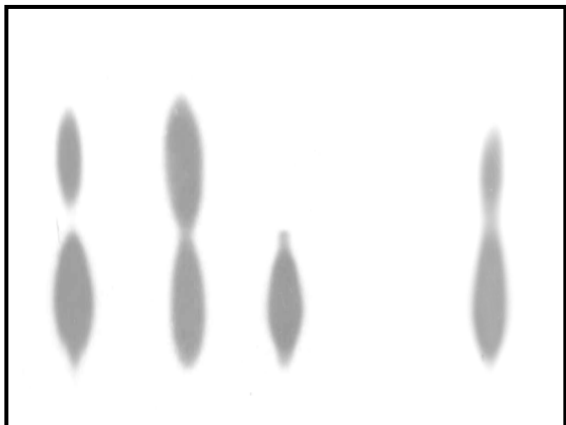


GTP Hydrolysis

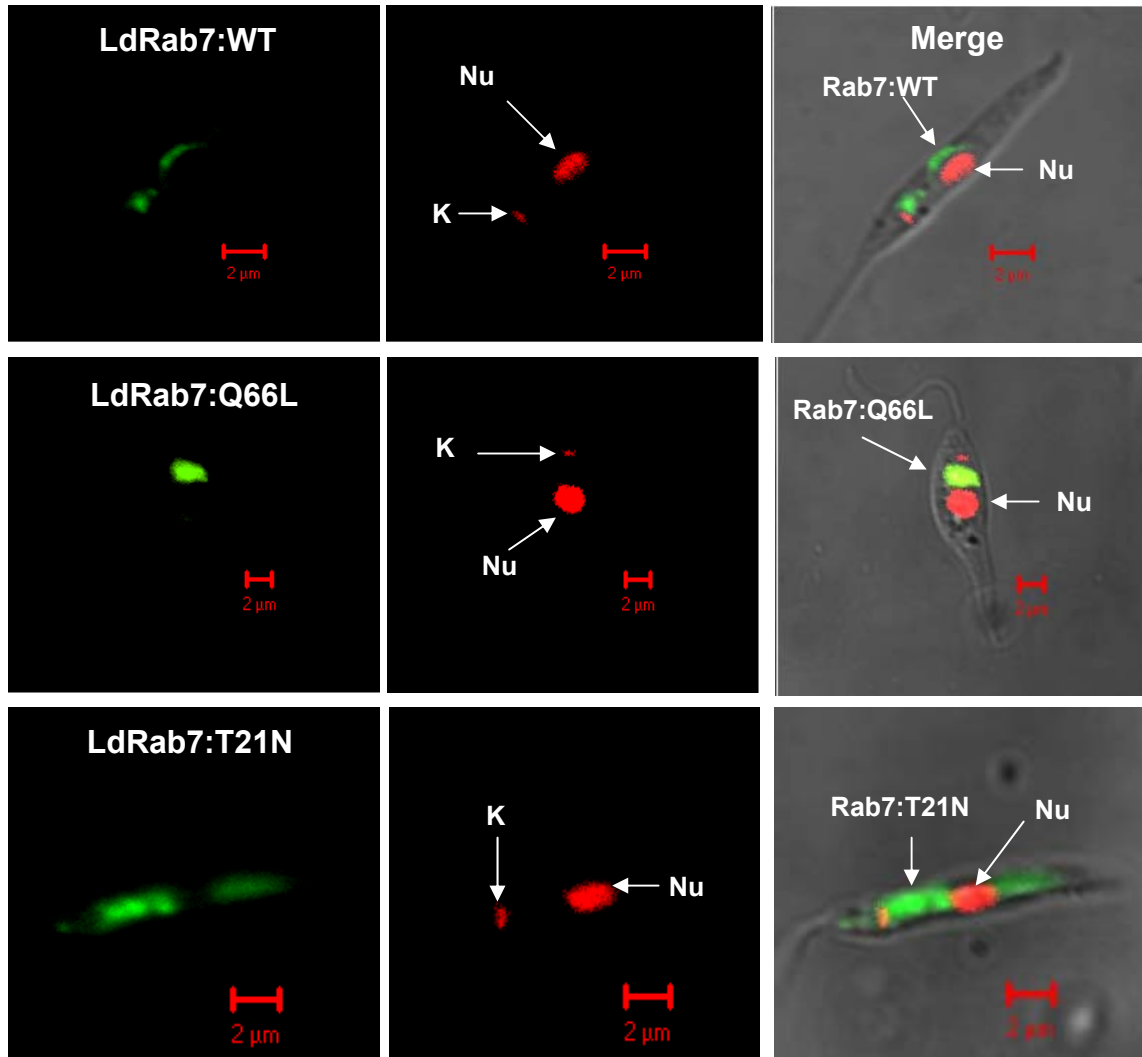
MRab7:WT
 LDRab7:WT
 LdRab7:Q66L
 LdRab7:T21N
 LdRab7:ΔC4

GDP

GTP

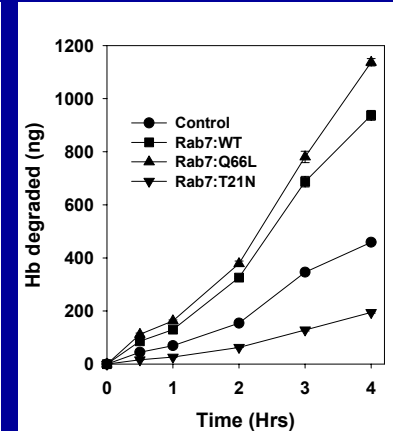
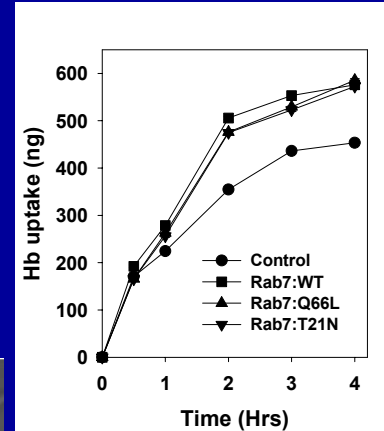
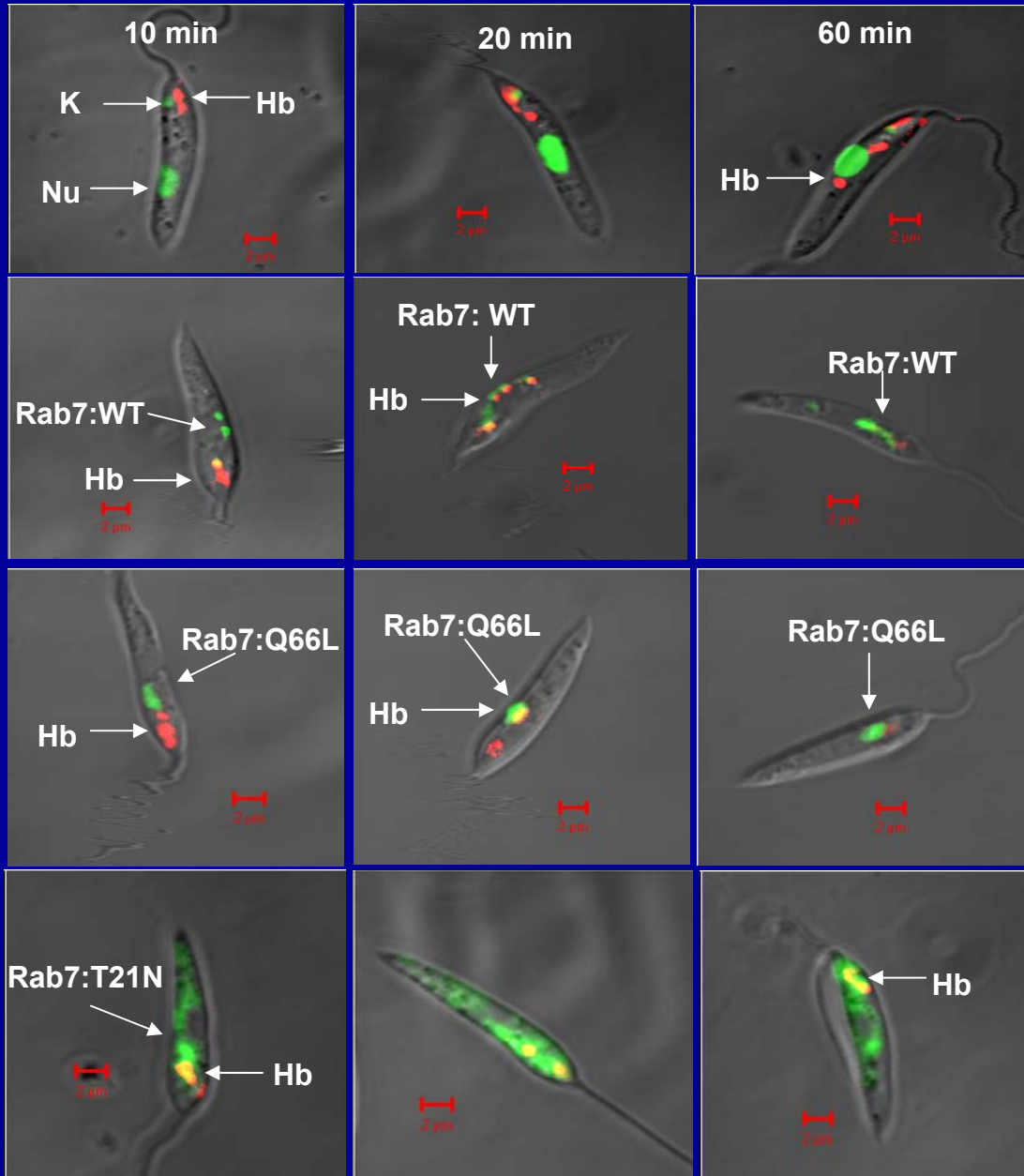


Characterization & localization of different mutants of Rab7 in *Leishmania*



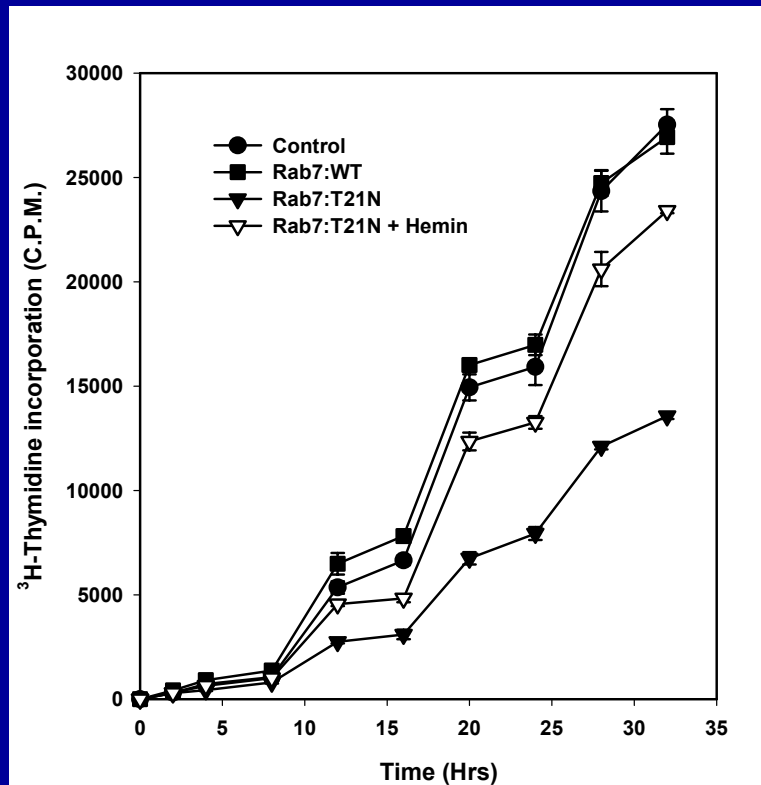
Regulation of hemoglobin trafficking by Rab7 in *Leishmania*

Uptake and degradation of ¹²⁵I-Hb By Rab7 overexpressed *Leishmania*



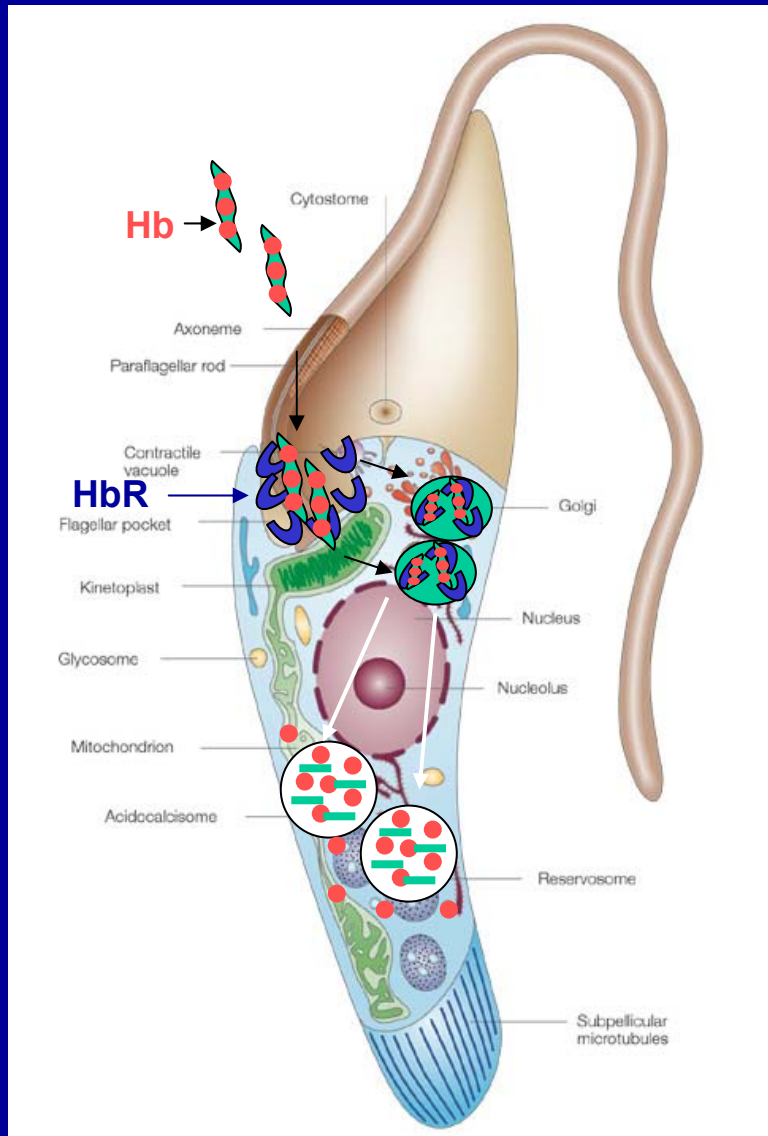
Rab7 in GTP form target the hemoglobin to the late lysosomal compartment for degradation.

Role of hemoglobin degradation on the growth of *Leishmania*



Inhibition of hemoglobin trafficking to the lysosomes in the cells over-expressing Rab7 negative mutant (T21N) compromised the growth of the *Leishmania* demonstrating that Rab7 mediated hemoglobin degradation to generate intracellular heme is necessary for the growth of *Leishmania*.

Hemoglobin endocytosis in *Leishmania*



Presence of specific Hb receptor in the flagellar pocket.

J. Biol. Chem. (1999) 274:2758

Hb binds with its receptor which is a surface localized hexokinase.

J. Biol. Chem. (2005) 280:5884

Bound Hb is internalized into early endosomal compartment by Rab5 regulated process.

EMBO J. (2003) 22:5712

Subsequently, Hb is targeted to the lysosomes where it is degraded to generate intracellular heme.

PNAS, USA. (2008) 105:3980.

I have shown you how endocytosis and intracellular trafficking is regulated in a unicellular protozoan pathogen through hemoglobin endocytosis in *Leishmania*. This receptor system appears to be a very significant process to acquire heme from the intracellular degradation of internalized hemoglobin by *Leishmania*. Inhibition of hemoglobin uptake or its intracellular degradation is found to be detrimental to the cells suggesting that HbR could be a novel therapeutic target for Leishmaniasis.

**Intracellular trafficking
In *Leishmania***

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Ganga Krishnamurthy
Senthil Kumar
Shruti Agarwal
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Surbhi Bhal
Sonali Bose
Deepika Gupta**

**Mechanism of
Phagocytosis**

**Konark Mukherjee
Shehla Hashim
Shadab Siddique
Parashuraman
Malabika Bhattacharya
Namrata Ohja
Richa Madan**

**Site specific drug
Delivery**

**Vikram Prasad
S. Sridhya
Saubiya Siddique**

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Thank you