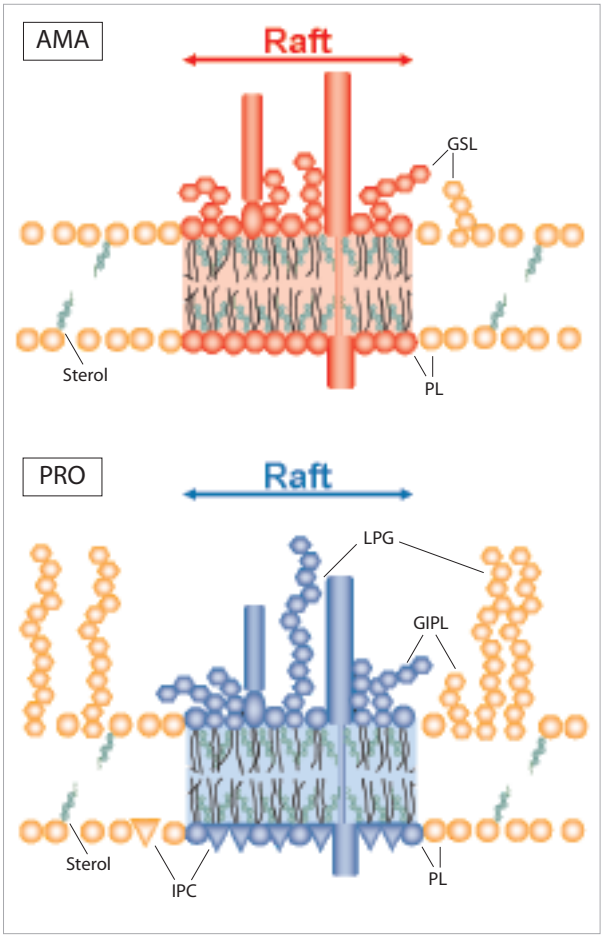
MEMBRANE MICRODOMAINS ENRICHED IN (GLYCO) (SPHINGO) LIPDS AND STEROLS: ORGANIZATION, FUNCTION AND CELL SIGNALING

Hélio Kiyoshi TAKAHASHI

Biochemistry Departament / Federal University of São Paulo (Unifesp)



Hypothetical membrane rafts in L. (L.) amazonensis. *Lateral association between sphingolipids and sterols results in formation of rafts. Transmembrane and GPI-anchored proteins may serve as platform for cell signaling. GSL, glycosphingolipid; IPC, inositol phosphorylceramide; LPG, lipophosphoglycan; PL, phospholipid; GIPL, glycoinositolphospholipid*

In the past 10 years our laboratory have isolated and characterized various glycolipid antigens from *Leishmania*, *Trypanosoma cruzi* and pathogenic fungi. Glycolipid antigens are mainly located at the plasma membrane of these microorganisms, and recent results have shown the involvement of these molecules in processes of cell adhesion, recognition, and differentiation. The major objective of this project is to study, in details, the organization of lipids and glycolipids in microdomains at the plasma membrane, denominated membrane rafts, and their role in processes of adhesion, invasion, and survival of fungi and parasites in the mammalian host. Also, molecules from the host cells, presumably involved in the interaction with these pathogens, will be characterized, and the activated signal pathways will be analyzed in order to better understand the pathogen-host interaction. The specific objectives of this project are focused at determining and characterizing: i) components of the microdomains enriched in (glyco)(sphingo)lipids in trypanosomatids and pathogenic fungi; ii) interaction of fungi and trypanosomatids, as well as its purified glycoconjugates, with mammal cells; iii) structure and function of specific antigens of parasites and fungi, aiming to identify new target molecules for action of more specific drugs; iv) *in vitro* and *in vivo* effects of inhibitors involved in the biosynthesis of lipids, in the growth and infectivity of parasites and fungi; v) the functional and organizational dynamics of the microdomains in the pathogenesis of these microorganisms; vi) reorganization of membrane rafts and cytoskeleton during phase transition in dimorphic pathogenic fungi; vii) processes of cellular signaling in the interaction of parasite-cell host and fungi-host cell; and viii) (glyco)lipidomic analysis of fungi and parasites aiming to detect possible virulence markers.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Membrane rafts are cholesterol – and sphingolipid – enriched cell membrane domains, which are ubiquitous in mammals and play an essential role in different cellular functions, including host cell-pathogen interaction. By using several approaches, as localization in cell membrane of GM1, a membrane raft marker our group demonstrated that epithelial cell membrane rafts are essential for *Paracoccidioides brasiliensis* adhesion and activation of cell signaling molecules such as SRC-family kinases (SFKs).

Membrane rafts are also present in fungi. We observed that membrane domain fractions from *Histoplasma capsulatum* showed an enrichment of glycoinositol-phosphorylceramides, ergosterol, and proteins, such as PMA1P, a known yeast membrane raft marker. In addition, it was verified that disruption of fungal membrane rafts promoted an inhibition by 40% of *H. capsulatum* infection in alveolar macrophages, suggesting that integrity of protein and lipid organization of *H. capsulatum* membrane rafts is essential for yeast-cell interaction.

In a similar fashion, membrane rafts were demonstrated to be present in amastigotes (AMA) and promastigotes (PRO) of *Leishmania (Leishmania) amazonensis*, and promastigotes of *Leishmania (Viannia) braziliensis*. Regarding sphingolipids, promastigote membrane rafts present inositol phosphorylceramide (IPC) whereas membrane rafts of amastigotes of *L. (L.) amazonenis* present neutral glycosphingolipids. The disruption of these microdomains by incubating parasites with methyl-β-cyclodextrin inhibited significantly macrophage infectivity by *Leishmania*. Also the role of parasite sphingolipids in cell cycle, division and viability was analyzed by employing inhibitors of IPC synthase (Aureobasidin A, AbA) and serine palmitoyl synthase (myriocin). Aba completely inhibited growth of amastigote and promastigote forms of *L. (L.) amazonensis*, and myriocin promoted parasite cytokinesis inhibition. Taken together, these results indicate that (glyco)(sphingo)lipids are key molecules in the processes of invasion and infection of trypanosomatids and fungi.

MAIN PUBLICATIONS

Maza PK, Straus AH, Toledo MS, Takahashi HK, Suzuki E. 2008. Interaction of epithelial cel membrane rafts with *Paracoccidioides brasiliensis* leads to fungal adhesion and SRC-family kinase activation. *Microbes and Infection*. DOI: 10.1016/j.micinf.2008.02.004

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Hélio Kiyoshi TAKAHASHI

Universidade Federal de São Paulo (Unifesp)
Departamento de Bioquímica
R. Botucatu, 862 – Vila Clementino
04523-900 – São Paulo, SP – Brasil

+55-11-5576-4502
takahashi.bioq@epm.br