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Entamoeba histolytica: Biochemical and molecular insights into the activities within microsomal fractions ☆

Milena Salgado [a], Julio C. Villagómez-Castro [b], Rocío Rocha-Rodríguez [b], Myrna Sabanero-López [b], Marco A. Ramos [a,1], Alejandro Alagón [a], Everardo López-Romero [b], Rosana Sánchez-López [a,*]

[a] *Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología (UNAM), Avenida Universidad 2001, Cuernavaca, Morelos 62210, Mexico*
[b] *Instituto de Investigación en Biología Experimental, Facultad de Química, Universidad de Guanajuato, Apartado Postal 187, Guanajuato, Guanajuato 36000, Mexico*

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Abstract

One of the most fascinating aspects of the *Entamoeba histolytica* trophozoite ultrastructure is the lack of a typical secretory pathway, particularly of rough endoplasmic reticulum and Golgi system, in a cell with such a high secretory activity. Here, we describe the isolation of amoeba cell structures containing ER-typical activities. Following isopycnic centrifugation of plasma membrane-free extracts, microsomes enriched in enzymatic activities such as dolichol-*P*-mannose synthase (DPMS; EC 2.4.1.83), UDP-GlcNAc:dolichol-*P* GlcNAc-1-*P* transferase (NAGPT; EC 2.7.8.15), and UDP-D-GlcNAc:dolichol-*PP* GlcNAc (NAGT; EC 2.4.1.141) were resolved from phagolysosomal fractions. Sec61α-subunit, an ER-marker involved in the translocation of nascent proteins to the ER, was found to co-fractionate with DPMS activity indicating that they are contained in microsomes with a similar density. Further, we optimized conditions for trophozoite homogenization and differential centrifugation that resulted in the separation of a 57,000*g*-sedimenting microsomal fraction containing *Eh*Sec61α-subunit, *Eh*DPMS, and *Eh*PDI (protein disulfide isomerase, a soluble marker of the lumen of the ER). A relevant observation was the lack of ER markers associated to the nuclear fraction. Large macromolecular structures such as *Eh*proteasome were sedimented at a higher speed. Our knowledge of the molecular machinery involved in the biosynthesis of dolichol-linked oligosaccharide was enriched with the identification of putative genes related to the stepwise assembly of the dolichol-*PP*-$GlcNAc_2Man_5$ core. No evidence of genes supporting further assembly steps was obtained at this time.

Index Descriptors and Abbreviations: ALG, asparagine-linked glycosylation; AP, acid phosphatase; DPMS, dolichol-*P*-mannose synthase; ER, endoplasmic reticulum; Glc, glucose; GPI, glycosyl phosphatidylinositol; Man, mannose; β-NAcGase, β-*N*-acetyl-glucosaminidase; NAGPT, UDP-D-*N*-acetylglucosamine:dolichol-*P* *N*-acetylglucosamine-1-*P* transferase; NAGT, UDP-D-*N*-acetylglucosamine:dolichol-*PP* *N*-acetylglucosamine; PDI, protein disulfide isomerase; UDP-GlcNAc, UDP-*N*-acetylglucosamine; GFP, green fluorescent protein; DHFR, dihydrofolate reductase

Keywords: *Entamoeba*; Endoplasmic reticulum; Microsomes; Dolichol-linked; Oligosaccharide; N-Glycosylation

1. Introduction

Eukaryotic cells contain highly specialized, membrane-bounded compartments fulfilling specific functions. Among them, the endomembrane system that constitutes the protein trafficking and the lipid biosynthetic pathways is the most prominent. It consists of the

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* Corresponding author. Fax: +52 777 3172388.
E-mail address: rosana@ibt.unam.mx (R. Sánchez-López).
[1] Present address: Facultad de Ciencias Químicas e Ingeniería, UABC, Calzada Tecnológico 14418, Tijuana, BC 22390, Mexico.

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endoplasmic reticulum (ER), pre-Golgi intermediates, the Golgi apparatus, and different types of post-Golgi carriers and vesicles. The protein transport pathway accomplishes a multitude of interrelated functions including the translocation to the ER and transport of de novo synthesized proteins, the N-linked glycosylation and sorting of proteins to their final cellular destinations, such as the lysosome, endosome, plasma membrane, and extracellular environment.

The first events of protein N-linked glycosylation take place in the ER. Accordingly, biosynthesis of the nucleotide-activated sugar precursors as well as the early steps of assembly of the dolichol-linked oligosaccharide occur at the cytoplasmic side of the ER membrane whereas the further addition of four mannose and three glucose residues takes place in the lumen of the ER (Burda and Aebi, 1999). This results in the formation of dolichol-*PP*-$GlcNAc_2Man_9Glc_3$ intermediate which is transferred en bloc by the oligosaccharyltransferase complex (OST) to a selected Asn-X-Ser/Thr sequence of nascent polypeptides (Knauer and Lehle, 1999). N-Linked oligosaccharide is further modified and extended by the removal and addition of sugar residues along the ER and Golgi transport to generate different forms of *N*-glycans (Dean, 1999).

Entamoeba histolytica, the protozoan parasite responsible for human amebiasis, is estimated to cause severe disease in 48 million people, killing 70,000 each year [WHO/PAHO/UNESCO, 1997]. Since the early studies of *Entamoeba* cell biology, it became quite fascinating the fact that the nucleus is the only organelle identified by electron microscopy. A cytosol abundant in apparently undifferentiated vesicles and vacuoles of various sizes but lacking other typical eukaryotic organelles such as mitochondria, Golgi, and rough endoplasmic reticulum (rER) characterizes the ultrastructure of this parasite (Bakker-Grunwald and Wostmann, 1993; Carrero and Laclette, 1996). Nevertheless, rER- and Golgi-like functions seem to be present in the trophozoite. Amoebal virulence has been associated to the active transport to the cell membrane of the Gal/GalNAc inhibitable lectin (Gilchrist and Petri Jr., 1999) and the secretion of cystein-proteases and amoebapore, among others (García-Rivera et al., 1999; Que and Reed, 1997; Leippe, 1997). Studies on the effect of tunicamycin on trophozoites confirmed N-glycosylation of the Gal/GalNAc inhibitable lectin (Mann et al., 1991). O-Glycosylation as well as the synthesis of GPI-anchored proteins have also been reported (McCoy et al., 1993; Moody-Haupt et al., 2000; Stanley Jr. et al., 1995). Brefeldin A-sensitive and -insensitive protein transport mechanisms seem to be present in *E. histolytica* trophozoites suggesting two different secretory pathways (Gosh et al., 1999; Manning-Cela et al., 2003). Despite all this evidence, amoebal subcellular structures fullfilling ER- and Golgi-like functions have not been conclusively demonstrated nor isolated or characterized.

Two independent approaches were undertaken by our groups to investigate the existence of ER-functional structures in *E. histolytica* trophozoites. On one hand, enzymatic activities involved in the early reactions of the dolichol-linked oligosaccharide assembly were assessed. Accordingly, detergent-solubilized membranes of *E. histolytica* trophozoites displayed activities of DPMS, NAGPT, and NAGT (Vargas-Rodriguez et al., 1998; Villagómez-Castro et al., 1998). Another ER activity presumptively involved in the *N*-glycan processing, such as type II-like α-glucosidase, which is responsible for the removal of two α-1,3-linked glucosyl residues of the $GlcNAc_2Man_9Glc_3$ oligosaccharide in yeast and animal cells (Hersocovics, 1999a,b) was also detected in amoeba membranes (Zamarripa-Morales et al., 1999) and later purified and characterized (Bravo-Torres et al., 2004).

On the other hand, entamoebal genes coding for highly conserved eukaryotic ER and Golgi resident proteins were cloned, thus providing the first molecular evidence of functions related to these organelles in *E. histolytica* (Sánchez-López et al., 2000a). These included *Eh*SRP54, which codes for a subunit of the SRP complex involved in the first step of the secretory pathway, and the *Eh*ERD2 ortholog responsible for the retrieval of ER luminal proteins from post-ER compartments (Ramos et al., 1997; Sanchez-Lopez et al., 1998). More recently, we have reported the isolation of the genes coding for a protein disulfide isomerase (*Eh*PDI), a chaperone-like resident of the lumen of the ER, which catalyzes the formation, breakage, and rearrangements of disulfide bonds in nascent proteins (Ramos and Alagón, 2000), *Eh*STT3, a subunit of the OST complex (Gutiérrez et al., 2000), and *Eh*Sec61α-subunit, a core component of the ER protein translocation machinery (Sánchez-López et al., 2000b), respectively.

Here, we provide strong biochemical and molecular evidences supporting the presence of the machinery required for N-linked glycosylation of nascent proteins in *E. histolytica*. Results of subcellular fractionation indicate that some of the enzymes involved in the dolichol pathway and the ER molecular markers *Eh*Sec61α-subunit, *Eh*DPMS and *Eh*PDI are all contained in membrane compartments with similar density.

2. Materials and methods

2.1. Strains and culture conditions

Trophozoites of *E. histolytica*, strain HM1:IMSS, were maintained and propagated under axenic conditions in the TYI-S-33 medium at 37 °C (Diamond et al., 1978).