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Entamoeba histolytica: intracellular distribution of the sec61α subunit of the secretory pathway and down-regulation by antisense peptide nucleic acids

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Abstract

The Sec61α protein is defined as a highly conserved essential integral component of the endoplasmic reticulum in eukaryotic cells. We report a detailed immunolocalization of the *Entamoeba histolytica* homologue of the Sec61α subunit (*Eh*Sec61α), which shows an irregular pattern throughout the cell and is also found on the cell surface, its effective down-regulation by means of antisense peptide nucleic acids and its effects on cell proliferation, subcellular distribution of two virulence factors, and the ability of the trophozoites to cause liver abscess in hamsters. Although Sec61α levels are specifically decreased in antisense PNA-treated trophozoites, which proliferate more slowly than the controls, mobilization of the cysteine protease 5 and amoebapore to the cell surface is not significantly impeded and the capacity to induce liver abscess in hamsters is largely unaffected. The implications of these findings are discussed in the context of the peculiar cell biology of *E. histolytica*.

Index Descriptors and Abbreviations: Entamoeba histolytica; *Endoplasmic reticulum*; Sec61α subunit; Abscess; Antisense peptide nucleic acid; CP5, cysteine protease 5; ER, endoplasmic reticulum; PNA, peptide nucleic acid

1. Introduction

The *Entamoeba histolytica* trophozoite, the etiologic agent of amebiasis in humans, is a motile, fluid, and actively phagocytic and secretory cell. One of the most salient characteristics of *E. histolytica* is the apparent lack of fixed structural compartments identifiable by microscopy. In fact, *E. histolytica* has often been described as devoid, among other things, of intracellular compartments morphologically equivalent to the Endoplasmic Reticulum (ER) and Golgi system (Martínez-Palomo, 1986), which are responsible for an important part of post-translational modification and protein traffic in model systems such as yeast and mammalian cells. Although the *E. histolytica* cell is very atypical by comparison to other eukaryotes, in the last few years evidence, both biochemical and genetic, has been accumulating which demonstrates the existence of post-translational protein modification. Thus, some enzymatic activities associated to post-translational modification of proteins have been characterized (Vargas-Rodríguez, 1998; Villagómez-Castro et al., 1998), and an assortment of genes closely homologous to components of the secretory pathway of model eukaryotes have been cloned and

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sequenced (Gutiérrez et al., 2000; Juárez et al., 2001; Ramos and Alagón, 2000; Ramos et al., 1997; Saito-Nakano et al., 2000; Sanchez-Lopez et al., 2000). Furthermore, a recent study established that Brefeldin A (BFA), which in other systems inhibits Golgi-dependent transport, partially affects adhesion of trophozoites to a fibronectin substrate as well as several exocytic and transport processes in vitro. The authors conclude that two mechanisms, one akin to the BFA-sensitive 'classical' vesicular transport and another alternate, inducible, BFA-insensitive system, coexist in *E. histolytica* (Manning-Cela et al., 2003). All these data contribute to a growing body of evidence available today which points to the existence of protein traffic and sorting systems functionally equivalent to those of other, better studied, eukaryotes. They also indicate that at least some important aspects of protein traffic and processing are likely to differ significantly from textbook model systems.

In an effort to understand the spatial organization of protein traffic in *E. histolytica*, our laboratory has, over the last few years, cloned and analyzed the intracellular distribution of several ER and Golgi marker genes highly homologous to those of model organisms. One such gene, reported by Sanchez-Lopez et al. (2000), is the homologue of the *SEC61* gene of *Saccharomyces cerevisiae*, or Sec61α in mammals, which codes for a key structural component of the translocation system known as the translocon, which has been the object of intense studies (reviewed in Eichler and Duong, 2004). Functional studies in this multiprotein system, in which Sec61α is thought to conform an aqueous pore, suggest that it is responsible for the translocation of nascent proteins with signal peptides into the ER by a complicated series of interactions between the nascent peptide–mRNA–ribosome complex, the signal recognition particle (SRP) complex itself composed of several proteins and an RNA scaffold (Walter and Blobel, 1980; Walter and Johnson, 1994) and the translocon complex, which culminates with the cotranslational entry of the peptide into the lumen or the membrane of the ER, where it will be further modified and continue to subsequent destinations within the cell or be secreted. In *S. cerevisiae*, the *SEC61* gene is essential, presumably because its absence severely hampers protein traffic since the translocon is thought to be an obligatory early point of entry into the protein modification and sorting systems (Stirling et al., 1992).

In mammalian cells, a detailed study of cytolocalization of Sec61α revealed that although most of the signal could be found in the perinuclear ER and ER-Golgi intermediate compartment (ERGIC), as could be reasonably expected, a diffuse reticular labeling throughout the cells was also evident. The authors concluded that Sec61α is recycled from more distal compartments back into the ER-ERGIC although no known localization signals have been detected in Sec61α (Greenfield and High, 1999). This study did not pursue the question of whether Sec61α (and the other markers they examined) are functionally involved in protein translocation in these distal compartments, or whether they are part of non-functional structures awaiting recycling.

In the amitochondriate protozoan parasite *Giardia intestinalis* distinct compartments can be revealed by the use of ER molecular markers, both in proliferating as well as encysting *Giardia* trophozoites. A similar pattern, with strong perinuclear signals progressively weakening toward the cellular periphery could be observed (Marti et al., 2003a,b). Although the nature of the Golgi in this cell—in terms of its origin, structure and functionality in developmental stages—still remains the subject of debate, it is quite clear that an equivalent of the ER exists and can be defined spatially and functionally. In the intracellular Microsporidia, the eukaryote with the smallest genome, genes coding for two subunits of the Sec61 complex are found, pointing to the ubiquity and conservation of the *SEC61* gene group (Beznoussenko Mironov, 2002).

In cells in which a rough endoplasmic reticulum (RER) has been described, most notably higher animal cells, there is an evident spatial coupling of mRNA export and translation/translocation, as translation of the exported mRNA occurs at, or in the near vicinity of, the outer leaflet of the ER boundary, maximizing the probability of translocation of nascent peptides with appropriate signal sequences across the translocon of the ER-cytosol interface, and the initiation of their transit through the ER- and Golgi-dependent pathways. In the case of *Giardia*, the strong perinuclear labeling which fades toward the cellular periphery suggests a similar arrangement. Thus, reasonably fixed spatial relationships condition the functional relationships between successive compartments. In the case of *E. histolytica*, the equivalent of the RER and fixed compartments have not been described despite detailed electron microscopy studies, and whether they are altogether absent or their structure is very labile remains a very open question, and one that has important implications for the functionality of the secretory pathway in terms of the models that may be devised or used to understand it (Beznoussenko Mironov, 2002; Storrie and Nilsson, 2002) and to the life circumstances, the cellular physiology and pathogenic potential of *E. histolytica*.

Ulceration and destruction of host tissues, notably the colonic epithelium and the liver, resulting from infection by *E. histolytica* are thought to be the result of interactions between parasite factors and host factors. *E. histolytica* factors that influence the ability of the trophozoite to invade and cause tissue damage include, among others, pore-forming peptides (amoebapore; Leippe, 1997), a galactose *N*-acetylgalactosamine (Gal-GalNAc)-specific lectin (Petri et al., 2002) and a family of at least seven cysteine proteases (Que and Reed, 1997). Although the mechanism of invasion is far from being understood, several studies, particularly those using