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Molecular characterization of *Entamoeba histolytica* RNase III and AGO2, two RNA interference hallmark proteins

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Abstract

Entamoeba histolytica, a protozoan parasite with variable DNA content and complex ploidity, has defied most efforts aimed at gene depletion using classical genetic methods. In this study, we identified and characterized two proteins involved in the RNA interference (RNAi) pathway, RNase III and AGO2. Our results strengthen the findings that an RNAi pathway does exist in this parasite.

Index Descriptors and Abbreviations: RNAi, RNA interference; siRNA, small interfering RNA; dsRNA, double strand RNA; Gal4AD, Gal4 activation domain; Gal4BD, Gal4 DNA binding domain

Keywords: *Entamoeba histolytica*; RNase III; AGO2; RNA interference

Entamoeba histolytica, a protozoan parasite causing an intestinal disease called amoebiasis, accounts for around 50 million intestinal infections in humans worldwide that result in 100 thousand deaths annually. Therefore, elucidation of the factors and genes that determine the nature of the relationship between the parasite and its host is of great interest. A common way to determine a gene's function is to inactivate the gene, and then examine the resulting phenotype. Yet, several features of *E. histolytica trophozoites*, like their variable DNA content and complex ploidity, have made it difficult to perform classical genetic studies aimed at the characterization of a genes function by inactivation.

One approach that has been shown to successfully inactivate genes in *E. histolytica* is the antisense strategy. Yet, despite being a very time costly approach, this method has been reported to efficiently downregulate only a small number of genes, namely cysteine proteases (Ankri et al., 1998), lectins (Ankri et al., 1999), calcium binding protein (Sahoo et al., 2003), and alcohol dehydrogenase 2 (Espinosa et al., 2001).

RNA interference (RNAi), defined as the mechanism through which gene specific dsRNA triggers degradation of homologous transcripts, has taken the scientific world by storm by proving to be a tool of immense value to analyze gene function, especially in organisms that are not amenable to classical genetics like *E. histolytica*.

First reported in *Caenorhabditis elegans* by Mello and Fire (Fire et al., 1998), today this phenomenon has been described in almost all eukaryotic organisms, and more interestingly in protozoan parasites like *Trypanosoma brucei* (Shi et al., 2000), *Plasmodium falciparum* (Malhotra et al., 2002), *Toxoplasma gondii* (Al-Anouti et al., 2003), and *Paramecium* (Bastin et al., 2001). Two recently published studies have described the successful application of RNAi in *E. histolytica* in downregulating the expression of γ-tubulin (Vayssie et al., 2004) and diaphanous genes (Kaur and Lohia, 2004). In this study,

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we set out to characterize several components of an RNAi pathway in *E. histolytica* by examining the parasite's genetic makeup.

The RNAi pathway can be divided into two primary stages. First, dsRNA, derived from endogenous, transgenic or viral transcripts, is cleaved into 20–26 nt long small interfering RNAs (siRNAs), that have a 5′ phosphate and 3′hydroxyl termini and 2–3 nt 3′ overhangs, by RNase III comprising enzymes. RNase III enzymes can be divided into three classes based upon domain structure: bacterial RNase III which contains a single catalytic domain and a dsRNA binding domain, Drosha family nucleases containing dual catalytic domains, and Dicer enzymes which contain dual catalytic domains, and additional helicase and PAZ motifs.

We engaged in finding a gene candidate in the parasites genome that would code for an enzyme with RNase III activity. Database mining identified only one protein with an RNase III domain in the *E. histolytica* genome (Accession No. EAL45114) that showed 33% identity and 49% homology to the bacterial *Escherichia coli* RNase III enzyme (Fig. 1A). The protein sequence contains the nine amino acid conserved sequence: (D/E/Q)(K/R/Q/T)(L/M)E(F/Y/W)(L/V)GD(S/A/R/H) (Conrad and Rauhut, 2002) in the N terminal portion of the protein, which is characteristic of RNase III enzymes and supposedly forms the catalytic domain of the enzyme. Yet, the protein was not found to contain a dsRNA binding domain, usually found in the C-terminal portion of the protein. We then set out to determine if there was evident RNase III activity in lysates of *E. histolytica* trophozoite strain HM-1:IMSS that were grown under axenic conditions in Diamonds TYI-S-33 medium at 37 °C. dsRNA, corresponding to the gene sequence of *E. histolytica* DNA methyltransferase (Ehmeth) (Accession No. AY562533), was synthesized using the HiScribe

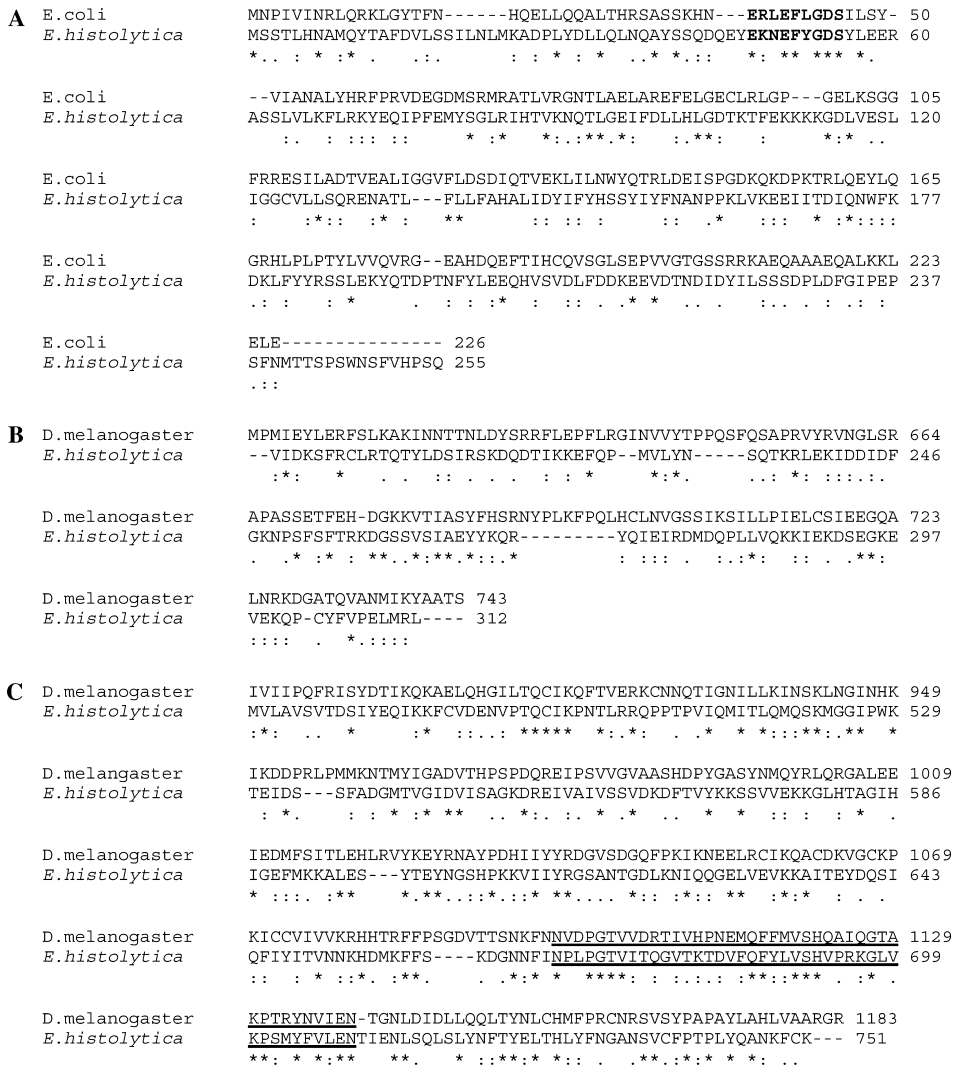

Fig. 1. Clustal alignment between (A) RNase III catalytic domain of *E. histolytica* RNase III and *E. coli* RNase III; (B) *D. melanogaster* and *E. histolytica* Paz, and (C) Piwi domain. In bold, catalytic domain of EhRNase III and *E. coli* RNase III. The underlined sequence represents the Piwi box that resides in the Piwi domain.