Available online at www.sciencedirect.com
ScienceDirect
Molecular & Biochemical Parasitology 152 (2007) 47–52
MOLECULAR & BIOCHEMICAL PARASITOLOGY

SINE elements of *Entamoeba dispar*☆

Abdirashid M. Shire *, John P. Ackers

Department of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Received 17 May 2006; received in revised form 31 October 2006; accepted 14 November 2006
Available online 8 December 2006

Abstract

Entamoeba histolytica and *E. dispar* are closely related protozoan parasites; the former causes clinical amoebiasis in humans while the latter appears to be non-pathogenic. The molecular biology of *E. histolytica* shows a number of unusual features, one of which is the abundance of polyadenylated but apparently untranslatable mRNAs produced; many of these are the product of at least three families of SINEs (EhSINE1–3). Here we show that the genome of *E. dispar* contains numerous copies of a SINE element (EdSINE1) whose 5′- and 3′-ends are very similar to those of EhSINE1 but with a much less similar middle portion. Twelve out of 18 copies examined had target site duplications. In none out of six cases examined was there a SINE element in the homologous region of the *E. histolytica* genome but a single copy of EdSINE1 is present in *E. histolytica* where it is identified as EhSINE3.

Keywords: *Entamoeba dispar*; *E. histolytica*; SINE; LINE; BspA-like protein

1. Introduction

Invasive amoebiasis (clinically manifested mainly as amoebic dysentery and amoebic liver abscess) remains one of the more significant human parasitic diseases with some 50 million new infections and possibly 40–100,000 deaths each year [1,2], largely in less developed countries. The diseases is caused by infection with the protozoan *Entamoeba histolytica*, although it is now clear that only a minority of those infected will develop clinical amoebiasis [3]. All other species of *Entamoeba*, and in particular the very closely related *E. dispar*, appear to be completely non-pathogenic [4].

The molecular biology of *E. histolytica* shows a number of unusual features, one of which is the abundance of polyadenylated but apparently untranslatable mRNAs produced [5]. Probably the most abundant of these transcripts are those first named IE [6,7]; the same sequences were subsequently described as *ehapt2* [8] and *EhLSINE1* [9]. The genomic IE/*ehapt2*/*EhLSINE1* sequences are abundant (250–500 copies per haploid genome), transcribed and polyadenylated (although no eukaryotic RNA polymerase promoters can be identified) but contain no plausible open reading frames [7].

In a study of a repetitive DNA sequence in *E. histolytica*, Sharma et al. detected the first non-LTR retrotransposon like element (EhRLE1) identified in this organism [10]. The high copy number and wide genomic distribution of the IE/*ehapt2*/*EhLSINE1* element make it likely that it is or was a mobile genetic element [9,11,12]. Strong support for this view comes from the observations that the ends of this element are flanked by short direct repeats of the target site and that a stretch of 74 nucleotides at the 3′-end of IE is nearly identical with the 3′-end of EhRLE1 [9]. This latter feature is seen in the SINE elements, which are non-autonomous and are thought to use the enzymatic machinery of LINEs (in this case EhRLE1) for their transposition [13].

It thus seems logical to refer to EhRLE1 as EhLINE1 and the IE/*ehapt2*/*EhLSINE1* sequences as EhSINE1s; Willhoeft et al. [12] could not detect *ehapt2* in *E. dispar* but the aim of this work was to see if sequences related EhSINE1s were present in the genome of this species.

Abbreviations: LINE, autonomous non-long-terminal-repeat retroposon (long interspersed repetitive element); SINE, short non-autonomous retroposon (short interspersed repetitive element); TSD, target site duplication

☆ *Note:* Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the accession no. DQ480085.

* Corresponding author. Present address: Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, 200 1st Street SW, Rochester, MN 55905, USA.
E-mail address: shire.abdirashid@mayo.edu (A.M. Shire).

0166-6851/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbiopara.2006.11.010

Table 1
Primers and PCR conditions

Primer	Sequence	Mg^{2+} (mM)	T_a (°C)	t_a (s)	t_e (s)	Cycles
IE1-5′	GCACGTCTGAAACACCACAC	1.5	55	30	90	30
IE1-3′	CTAGCTCAGGGGAGACTAAT					
INV5F	CATAACCAGTCCATAACTGG	1.5	55	30	90	30
INV5R	GTCTTAATCAGAGGGGACTC					
AEMH_5.2	TCTAAGGAAGGCAGCAGGC	1.8	59	30	30	30
AEMH_3.2	AAGGGCATCACACCTGTGTT					
H_SINE_1_F	ATTAGGGAATGCTGCAAAGG	1.8	61	30	30	30
H_SINE_1_R	GCCTTTGTTTGTTTTCTGCC					
D_SINE_1_F	GGGTCAAGTGGTTGAGGCTA	1.8	62	30	30	30
D_SINE_1_R	TTGCTTTTAGTTGTTGAACTGTGA					
H_SINE_3_F	TCAATTGATTGTTGAATATTTGGAA	1.8	61	60	120	35
H_SINE_3_R	TTGTGTTCTTCTTTTCCTAAACCC					

T_a: annealing temperature; t_a: annealing time; t_e: extension time.

2. Materials and methods

2.1. In vitro growth of E. dispar trophozoites and DNA extraction

Trophozoites of *E. dispar* strain SAW 760 were initially cultivated monoxenically in the presence of *Crithidia fasciculata* in YI medium [14] with 15% bovine serum. Cells were harvested after 72 h growth by centrifugation (300 × *g* for 5 m). The pellet was washed once with sterile phosphate buffered saline (PBS) and transferred to a 1.5 ml microcentrifuge tube. Genomic DNA was extracted using the CTAB method [15].

Later, axenic cultures of *E. dispar* SAW760 as well as axenic *E. histolytica* (strains HM-1, 200: NIH, Rahman and IULA:0593:1 [16]) all cultivated in LYI-S-2 medium [17] with 15% adult bovine serum, were used; DNA was extracted using the PUREGENE® DNA Purification Kit (Gentra Systems, Minneapolis, USA) following the DNA purification protocol for 1–2 million cells [PUREGENE 00960_1–2 million cultured cells] with addition of 1.5 μl of proteinase K solution (20 mg/ml) per 300 μl of suspension immediately following the addition of cell lysis solution (advice from Amber Volk, Gentra Systems).

2.1.1. Primers

Primers used in this study were designed with the help of Primer3 software [18] and are shown in Table 1. IE1-5′ and IE1-3′ primers were designed to amplify EhSINE1 and were based on the consensus 5′- and 3′-ends of these highly conserved elements [7]; as it turned out they also amplify EdSINE1. All primers were obtained from Sigma Genosys (Pampisford, UK).

2.1.2. PCR amplification

Amplification was by standard methods using *Taq* Polymerase from MBI Fermentas, Hanover, MD, USA (primer pairs IE1 and INV5) or BioLine UK and 1 μl of template DNA. Denaturation was at 94 °C for 1 min in all cases; annealing and extension parameters are shown in Table 1 and were followed by one final cycle of 72 °C for 5 min (IE1 and INV5) or 2 min.

All PCR products were separated in 1.2% agarose gels (Invitrogen) in 1× Tris-borate–EDTA buffer at 8.5 V/cm and visualized after staining with ethidium bromide. Size markers were ladders I and IV from BioLine.

2.1.3. Cloning and sequencing

PCR products were directly cloned into a pGEM-T Easy Vector (Promega Southampton, UK) according to the manufacturer's instructions. Plasmid DNA was purified using QIAprep miniprep kit (Quiagen Ltd., Crawley, UK) following the manufacturers directions. Plasmids were sequenced using the SP6 and T7 primers (MWG-Biotech Company London, UK).

2.1.4. Southern blot analysis

E. dispar genomic DNA was digested separately with *Eco*RI, *Dra*I and *Alu*I (MBI Fermentas) and fractionated on 0.8% agarose, transferred to a nylon membrane and probed with *E. dispar* IE1 PCR product (randomly labelled with [α-^{32}P]-dCTP using the Rediprime system and purified by S-200 microspin column, both from Amersham) under standard hybridisation conditions.

2.1.5. Northern blot analysis

The mRNA was isolated according to QuickPrep Micro-mRNA purification kit (Amersham). *E. histolytica* and *E. dispar* mRNA (2.5 μg) was analysed on a 1% agarose mini-gel and hybridised as above.

2.1.6. Chemicals and medium constituents

All were from Sigma–Aldrich, Poole, Dorset UK.

2.1.7. Bioinformatic analysis

Preliminary *E. histolytica* and *E. dispar* sequence data was downloaded from The Institute for Genomic Research (TIGR) through their website at http://www.tigr.org and BLAST searches [19] carried out locally and at the NCBI website (http://www.ncbi.nlm.gov/BLAST). Sequences were aligned using CLUSTAL W [20]. Sequence divergence was calculated and plotted using DnaSP version 4.0 [21]. Note that while much TIGR *Entamoeba* data has been deposited in the WGS division of GenBank this is largely smaller contigs. Accession