Available online at www.sciencedirect.com
ScienceDirect
Molecular & Biochemical Parasitology 158 (2008) 82–94
MOLECULAR & BIOCHEMICAL PARASITOLOGY

Trichostatin A regulates peroxiredoxin expression and virulence of the parasite *Entamoeba histolytica*☆

Elada Isakov[a], Rama Siman-Tov[a], Christian Weber[b,c], Nancy Guillen[b,c], Serge Ankri[a,*]

[a] *Department of Microbiology, Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology and the Rappaport Institute, Haifa, Israel*
[b] *Institut Pasteur, Unite de Biologie Cellulaire du Parasitisme, Paris F-75015, France*
[c] *INSERM U786, Paris F-75015, France*

Received 20 September 2007; received in revised form 4 November 2007; accepted 22 November 2007
Available online 4 December 2007

Abstract

Histone deacetylation is associated with a repressed chromatin state, and histone acetylase and deacetylase activities have been previously described in *Entamoeba histolytica*. To investigate their roles in the control of *Entamoeba* gene expression, the parasite was grown in 50 nM trichostatin A (TSA), an inhibitor of histone deacetylase. TSA enhanced the cytopathic and hemolytic activity of the parasite and its resistance to oxidative stress. We first focused our attention on peroxiredoxin, a protein previously associated with *E. histolytica* virulence and resistance to oxidative stress. We found that the expression of peroxiredoxin was increased after TSA treatment, but were unable to confirm that this was a direct consequence of histone modification at the promoter. By microarray analysis, we found that some other mRNAs encoding some other virulence factors, such as the galactose-inhibitable lectin small subunits, were also increased. The pattern of gene expression was surprisingly different from that previously described after treatment with 150 nM TSA.

Keywords: Trichostatin; *Entamoeba*; Peroxiredoxin

1. Introduction

The acetylation of the core histone N-terminal "tail" domains is now recognized as a highly conserved mechanism for regulating chromatin functional states. This mechanism is regulated by the opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylation neutralizes the positive charge on histones, which disrupts higher-order structures in chromatin, thereby enhancing access of transcription factors, transcriptional regulatory complexes, and RNA polymerases to promoter regions of DNA. Histone deacetylation restores a positive charge on lysine residues of core histones, which allows chromatin to condense into a tightly supercoiled, transcriptionally silent conformation [1]. It is now well documented that aberrant transcription (i.e., epigenetic modulation) of genes that regulate cellular differentiation, the cell cycle and apoptosis is due to altered expression or mutation of genes that encode HATs, HDACs, or their binding and recruiting partners. Such modifications are key events in tumor onset and progression [2].

In protozoan parasites, the role of histone acetylation has recently been highlighted. In *Giardia lamblia*, the expression of variant-specific surface proteins (VSGs) seems to be dependent on histone acetylation [3]. In the malaria parasite *Plasmodium*, histone acetylation regulates gene expression during erythrocyte development [4]. For *Toxoplasma gondii*, histone acetylation is a marker of gene activation during differentiation, and promoter regions of tachyzoite-specific genes have been shown to be hyperacetylated in tachyzoites, but hypoacetylated during the bradyzoite stage [5]. In *Trypanosoma brucei*, the HDACs DAC1 and DAC3 are essential and DAC4 is required for cell cycle progression [6]. Indeed, *T. brucei* displays unique histone modifications such as acetylation of the multiple lysines of the C terminus of histone H2A (TbH2A) [7].

The components of the epigenetic machinery of *Entamoeba histolytica* are currently being studied. 5-Methylcytosine (m5C)

☆ *Note*: GenBank accession numbers are XM_643430, XP_648509, XM_642922, XM_649403, and XM_651789.

* Corresponding author at: Department of Molecular Microbiology, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, P.O.B 9649, 31096 Haifa, Israel. Tel.: +972 4 829 5256; fax: +972 4 829 5225.
E-mail address: sankri@tx.technion.ac.il (S. Ankri).

0166-6851/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbiopara.2007.11.014

formation in repetitive DNA sequences and in heat shock protein 100 (Hsp100) is catalysed by Ehmeth, a protein that belongs to the DNA methyltransferase 2 family [8–11]. The presence of m5C in *E. histolytica* has recently been confirmed and extended to two virulence-related genes, cysteine proteinase and lysozyme [12]. We have previously shown that m5C in some specific repetitive sequences is sensed by a new protein called *E. histolytica*-methylated LINE binding protein EhMLBP [13].

The role of heterochromatin formation in the control of virulence gene expression in a number of pathogenic unicellular parasites has recently been reviewed [14]. Both HAT and HDAC activity has been detected in *E. histolytica* [15]. Interestingly, trichostatin A (TSA) [16] inhibits encystation of the reptile parasite *Entamoeba invadens*, which suggests that HDAC activity may have a role in parasite differentiation [17]. Recently, it has been reported that TSA (150 nM) induces the expression of encystation-related genes, such as the hypothetical protein 489.m00024 and heat shock proteins, that include Hsp70 [18], in *E. histolytica* strain 200:NIH. Indeed, several virulence-related genes, such as CP1 and the 35-kDa subunit of the Gal/GalNAc lectin are down-regulated by TSA in this strain [18].

Here, we report a quite different picture of the effect of TSA on gene expression in *E. histolytica*. TSA (50 nM) significantly enhanced *in vitro* virulence of HM-1:IMSS trophozoites. Peroxiredoxin and additional key genes participating in virulence, such as the light subunits of the Gal/GalNAc lectin, were identified among the genes that were up-regulated by TSA. The gene encoding Jacob, a protein involved in cyst formation, was also found to be activated by TSA. However, this phenotype did not fit with the regulation of other genes related to cyst formation, including stress-related proteins. The similarities and differences between the present study and that on strain 200:NIH [18] are discussed. This work emphasizes the role of epigenetic mechanisms in the control of *E. histolytica* virulence.

2. Materials and methods

2.1. Parasite and cell culture conditions

Trophozoites of the *E. histolytica* strain HM1:IMSS were grown under axenic conditions in Diamond's TYI-S-33 medium [19] at 37 °C. Trophozoites in the log phase of growth were used in all experiments. HeLa cells were maintained in continuous culture in T75 tissue-culture flasks in Dulbecco's modified Eagle's medium (DMEM) (Beth HaEmek) supplemented with 4 mM L-glutamine, 1 mM sodium pyruvate, penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% foetal calf serum. Cells were cultured at 37 °C in a humidified 5% CO_2 atmosphere.

2.2. Escherichia coli strains

The phenotypes of the *E. coli* strains used in this study were as follows.

XL1-Blue (Stratagene), recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac[F′proAB lacI^{q}ZDM15 Tn10 (tet^r)]; BL-21 (DE3) (Stratagene), *E. coli* B F$^-$ dcm ompT hsdS (r_B^- m_B^-) gal l (DE3).

2.3. Previously published methodologies

2.3.1. Cytopathic activity

The destruction rate of cultured HeLa cell monolayers by trophozoites grown with or without 50 nM TSA (Alomone Labs) for 48 h was determined as described previously [20,21]. Briefly, *E. histolytica* trophozoites were harvested by completely removing the existing medium (to remove cell debris, and dead and unbound trophozoites), adding fresh TYI-S-33 medium without serum, and chilling the tubes on ice. Following centrifugation at 700 × *g* for 10 min, the cell pellet was washed once with TYI-S-33 medium without serum, and viable trophozoites (5×10^5 ml^{-1}) were incubated with the HeLa cell monolayers.

2.3.2. Adhesion assay

Adhesion of trophozoites grown with or without 50 nM TSA for 48 h to HeLa cell monolayers was performed as previously described [21]. Trophozoites (2×10^5 ml^{-1}) used in this study were harvested as described in Section 2.3.1.

2.3.3. Haemolytic activity

The haemolytic activity of trophozoites grown with or without 50 nM TSA for 48 h was determined as previously described [22]. Trophozoites (2.5×10^5 ml^{-1}) used in this study were harvested as described in Section 2.3.1, except for the last wash, which was carried out in PBS.

2.3.4. Hydrogen peroxide killing assay

The resistance to oxidative stress induced by 2.5 mM *hydrogen peroxide* of trophozoites grown with or without 50 nM TSA for 48 h was determined as previously described [23]. Trophozoites were harvested as described in Section 2.3.1, and finally resuspended in DMEM supplemented with 70 mg L-cysteine and 135 mg ascorbic acid per 100 ml (DME-CH, pH 7.4) to a final concentration of 1×10^6 cells/ml. Trophozoites were incubated with 2.5 mM *hydrogen peroxide* for 1 h and their viability was determined by eosin exclusion.

Transfection of *E. histolytica* trophozoites was performed as described in [11].

ChIP analysis was performed as previously described [13] using 5 ml of an antibody against pan-Acetyl (SANTA CRUZ).

Preparation of nuclear acid-soluble proteins and acid/urea (AU) polyacrylamide gels were performed according to [24]. AU gels casted in a Mini-PROTEAN 3 cell (Bio-Rad) were used in this study. Nuclear-acid proteins where stained with the SilverQuest Silver staining kit (Invitrogen). This kit is compatible with mass spectrometry analysis.

2.4. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

Protein bands of interest were excised in gels digested with trypsin following a standard protocol [25] and analyzed by